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Zero field splitting fluctuations induced phase relaxation of Gd^{3+} in frozen solutions at cryogenic temperatures

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Abstract

Distance measurements using double electron–electron resonance (DEER) and Gd^{3+} chelates for spin labels (GdSL) have been shown to be an attractive alternative to nitroxide spin labels at W-band (95 GHz). The maximal distance that can be accessed by DEER measurements and the sensitivity of such measurements strongly depends on the phase relaxation of Gd^{3+} chelates in frozen, glassy solutions. In this work, we explore the phase relaxation of Gd^{3+} -DOTA as a representative of GdSL in temperature and concentration ranges typically used for W-band DEER measurements. We observed that in addition to the usual mechanisms of phase relaxation known for nitroxide based spin labels, GdSL are subjected to an additional phase relaxation mechanism that features an increase in the relaxation rate from the center to the periphery of the EPR spectrum. Since the EPR spectrum of GdSL is the sum of subspectra of the individual EPR transitions, we attribute this field dependence to transition dependent phase relaxation. Using simulations of the EPR spectra and its decomposition into the individual transition subspectra, we isolated the phase relaxation of each transition and found that its rate increases with $|m_s|$. We suggest that this mechanism is due to transient zero field splitting (*t*ZFS), where its magnitude and correlation time are scaled down and distributed as compared with similar situations in liquids. This *t*ZFS induced phase relaxation mechanism becomes dominant (or at least significant) when all other well-known phase relaxation mechanisms, such as spectral diffusion caused by nuclear spin diffusion, instantaneous and electron spin spectral diffusion, are significantly suppressed by matrix deuteration and low concentration, and when the temperature is sufficiently low to disable spin lattice interaction as a source of phase relaxation.

Keywords

Gd³⁺; Phase relaxation; Zero field splitting; W-band EPR; Spin labels

1. Introduction

The introduction of high-spin Gd^{3+} based spin labels (GdSL) into the practice of distance measurements by pulsed EPR seems highly promising, as demonstrated in a number of

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recent publications [1–9]. Since our first demonstration of Gd^{3+} – Gd^{3+} DEER (double electron–electron resonance) measurements on a model compound in 2007 [9], such distance measurements have been reported on model systems [8,9], peptides [6], proteins [4,7], nucleic acids [5] and coated nanoparticles [10]. Recently, the potential of W-band Gd^{3+} – Gd^{3+} DEER distance measurements in a membrane environment using trans-membrane model peptides has also been demonstrated [2]. These measurements featured high absolute sensitivity and showed that small changes in distances can be tracked. Distance measurements between a Gd^{3+} label and a nitroxide label have also been shown to be attractive in terms of sensitivity [10–15].

To further advance the application of this new spin labeling approach for distance measurements, optimization of a set of the interweaved external parameters (spectrometer frequency, pulse durations and their frequency difference, temperature, etc.) and internal parameters (zero field splitting (ZFS), phase and spin–lattice relaxation) should be carried out. These are particularly important in terms of signal-to-noise ratio (SNR), measurement time, distance of interest and amount of sample required. In recent publications [1,16], we addressed the issue of optimizing external parameters, while the internal parameters were treated only partially and empirically. Some of the reported findings [16] and other unpublished observations indicate that our current understanding of the fundamentals of one important internal parameter, the phase relaxation of GdSL, is insufficient. This parameter is crucial for DEER measurements because it determines the maximum accessible distances and affects the measurement sensitivity considerably. We observed that in addition to the usual mechanisms of phase relaxation known for radical based spin labels in general, and nitroxide based spin labels in particular, GdSL are subjected to an additional phase relaxation mechanism. This mechanism features an increase in the relaxation rate from the center to the periphery of the EPR spectrum. Considering the importance of phase relaxation in DEER, further advances in GdSL for distance measurements require an understanding of this additional mechanism, which would also provide new insights into optimizing sample preparation, chelate design and measurement adjustments.

In this paper, we explore the phase relaxation of GdSL. Because the EPR spectrum of GdSL is the sum of subspectra of the individual EPR transitions, we start by presenting experimental relaxation data, from which the phase relaxation kinetics of the individual EPR transitions are derived. This is followed by a discussion of possible sources of additional mechanisms of phase relaxation. Because spin lattice interactions affect phase relaxation, we also report measurements of spin lattice relaxation and its interpretation. We end by presenting a phenomenological approach where the dependence of the phase relaxation on concentration and solvent are reported. We chose as a representative for GdSL, Gd^{3+} -DOTA (Gd-DOTA, see Fig. 1), due to its small static ZFS [4] and its use as a Gd^{3+} label for DEER measurements.

2. Experimental

Sample preparation

Gd-DOTA solutions in the concentration range of 0.02–0.20 mM were prepared using the procedures described earlier [5,17]. In all cases, perdeuterated solvents were used. D_2O and

glycerol- d_8 were purchased from Cambridge Isotope Laboratories Inc., Andover, MA, USA and DMSO- d_6 was obtained from Sigma.

Spectroscopic measurements

All measurements were carried out at W-band (94.5–94.9 GHz) using a home-built W-band spectrometer [18]. This spectrometer has two independent mw-channels, which allow the generation of pulses having different durations and amplitudes. Echo detected EPR (ED-EPR) spectra were measured using a primary echo sequence of ϕ – τ – 2ϕ – τ –echo. The microwave power was set to give a nominal flip angle of $\phi = \pi/2$ at the central transition for a pulse length of 30 ns and τ was fixed at 500 ns. The phase relaxation kinetics were collected by recording the primary echo amplitude as a function of τ . In these measurements, the nominal $\pi/2$ pulse duration was 12.5 ns. At fields other than the central transition, the microwave power was adjusted to maximize the echo amplitude while keeping the parameters above constant. Some measurements were collected with a $\pi/2$ pulse of 30 ns. In all primary echo measurements, a standard two-phase cycle was applied to eliminate baseline offset. It was observed that in measurements, which require a time base exceeding 12–13 μ s, the phase of the output echo signal sometimes varied substantially due to some features of the ‘Quinstar’ power amplifier. When a larger time base was required, the measurements were performed point by point using manual phase correction to achieve a maximum echo amplitude. The measurements were performed in the temperature range of 6–25 K. At higher temperatures, spin lattice interactions begin to affect the phase relaxation substantially while at lower temperatures temperature stabilization was more difficult. The relaxation measurements were performed at a number of magnetic field positions over this temperature range. Longitudinal relaxation measurements were performed using the standard inversion recovery sequence with echo detection pulses of $2\pi/3$ each. The duration of the inversion pulse (efficiently a π pulse) was 15 ns as determined by means of a nutation experiment. The duration of the observation pulses was 60 ns. The initial separation, t_0 , between the inversion and observation pulses was 1000 ns and the separation between observation pulses was 500 ns.

3. Results and discussion

3.1. Phase relaxation – initial data and preliminary discussion

Fig. 2a presents a set of echo decay kinetics of 20 μ M Gd-DOTA collected at 10 K at various selected positions over the EPR spectrum. The ED-EPR spectrum is presented in Fig. 2b and arrows indicate the field positions at which the measurements shown in Fig. 2a were performed. The kinetics illustrate the magnitude of the effects of the extra relaxation mechanism on phase relaxation. The data obtained for other temperatures are similar in behavior and will be discussed in due course.

As evident from Fig. 2a, the kinetics of phase relaxation substantially changes over the EPR spectrum. The magnitude of the decay at a time base of ~ 12 μ s differs by about of e^2 times for kinetics collected at the center and at the far periphery of the EPR spectrum.

The dependence of phase relaxation on the position of the measurement in the EPR spectrum in solids (glasses) is a common fact known now for half a century. For instance, the rate of instantaneous diffusion (*iD*) and (electron spin) spectral diffusion (*eSD*) depend on the spin spectral density that is a function of the location of the measurement within the EPR spectrum. [19] In the case of a Gd^{+3} spectrum, the relaxation rate due to these two mechanism has to decrease from the center to the periphery because of the narrower spectrum of the central transition, which is the opposite of our experimental observation. Moreover, at a concentration of 20 μM and time base of $\sim 12 \mu\text{s}$, the echo decay due to *iD* does not exceed 2% at the pulse parameters utilized, and is negligible for *eSD*. The estimates of decays due to these mechanisms can be found in [5,16,20] and in the Supplementary Information (SI).

The field dependence of phase relaxation in glasses could be due to ‘small angle librations’ of a hyperfine **A**-tensor (*hfi*), as reported for nitroxide spin labels in [21–23]. The GdSL has 8–9 ligands in the first coordination sphere and the axes of *hfi* tensors of these ligands’ nuclei are directed at various angles relative to the external magnetic field. Overlapping numerous phase relaxation processes for different rates at given field positions should obscure the dependence of phase relaxation on the observation position, even if it could exist for each particular ligand *hfi* tensor in the first place. One can consider, however, the field dependence of phase relaxation from a different point of view. The EPR spectrum of GdSL is the sum of the subspectra of the various transitions and the observed effect could be not just field dependent, but spin state (m_s) dependent. In solids, two such m_s dependent phase relaxation mechanisms are known; spectral diffusion caused by nuclear spin diffusion (*nSD*) [24–29] and the indirect effect of spin lattice relaxation *via* accelerating intrinsic nuclear spin lattice relaxation [30]. As follows from the detailed discussion presented in SI, the first mechanism results in a dependence on m_s , which is the opposite from that observed in the experiment, while the second one is quite inefficient.

We have thus encountered a situation whereby none of the known mechanisms of phase relaxation in solids are able to explain the field dependence (m_s) of the experimental data. In liquids, however, such a mechanism is well known. The time dependent fluctuations of the ‘transient’ or ‘dynamic’ [31–33] zero field splitting (*tZFS*) provide an efficient, transition-dependent electron spin relaxation of Gd^{+3} . The origin of *tZFS* fluctuations in liquids is translational and rotational diffusion of ligands coordinated to Gd^{+3} as well as vibrations or ‘tumbling’ and ‘wobbling’ of ligands in the first coordination sphere. Having no other options at hand, we made an attempt to adapt such a mechanism to the relaxation of Gd^{+3} in glasses at cryogenic temperatures, understanding that such an adaptation would require redefining the magnitude and apparent origin of the *tZFS* and correlation times. A similar approach has been successfully used to explain nuclear quadrupole relaxation in solids [34,35], the mechanism of which is similar to *tZFS* driven relaxation.

The apparent consequence of this assumption, that phase relaxation kinetics is transition dependent, is that experimental kinetics collected at various fields are composed of contributions of the intrinsic kinetics of the various individual transitions. The first step in understanding the details of the transition-dependent phase relaxation is, therefore, to extract the intrinsic kinetics of each transition from the experimental data set. This undertaking

starts with the deconvolution of the EPR spectrum of GdSL into sub-spectra for the various transitions as described next.

3.2. Decomposition of ED-EPR spectrum

The EPR line shape of Gd^{3+} in frozen solutions is well understood [36,37]; it consists of a narrow, intense central line ($\langle -1/2 \rangle \leftrightarrow |1/2 \rangle$ transition) and a broad background signal produced by all of the other transitions. The Gd-DOTA spectrum has been previously measured at D-band (~ 135 GHz) [38], and simulated using ZFS tensors calculated in the framework of the modified ‘superposition’ model [38–40], according to which the ZFS tensor is the tensorial sum of the individual ZFS tensors of the ligands. Within the framework of this model, the ZFS parameter, D_Z , of Gd-DOTA was found to have a bi-modal distribution, which was well emulated by two Gaussians centered at $\pm D_c = 24$ mT with a width of $D = |D_c|/2$. Simulation of the W-band spectrum presented in Fig. 3 gave $D_c = 23$ mT and $D = 13$ mT, in reasonable agreement with the D-band results [38]. The transition-dependent spin-echo nominal turning angles have also been incorporated in the simulations as scaling factors, assuming that nominal $\pi/2$, π pulses were applied to the central transition [16].

The sub-spectra of the various transitions, shown in Fig. 4, allow evaluating the signal composition at any field position within the spectrum. We will, therefore, divide the spectrum into three magnetic field ranges: for $|B_0| > 120$ mT ($B^{(3)}$), the observed signal is solely due to the ($\langle \pm 7/2 \rangle \leftrightarrow |\pm 5/2 \rangle$) transitions; for $120 \text{ mT} > |B_0| > 60$ mT ($B^{(2)}$), the observed signal is a superposition of the ($\langle \pm 7/2 \rangle \leftrightarrow |\pm 5/2 \rangle$) and ($\langle \pm 5/2 \rangle \leftrightarrow |\pm 3/2 \rangle$) transitions, etc. It is also evident that the signal from the ($\langle -1/2 \rangle \leftrightarrow |1/2 \rangle$) transition is localized within ± 5 mT of the center ($B^{(0)}$). At the maximum of the EPR spectrum, the signal intensity of the central transition is about an order of magnitude greater than the sum of the intensities from all of the other transitions. Now, that we have decomposed the ED-EPR spectrum into its individual transitions we can proceed to analyze the phase relaxation in terms of the individual EPR transitions, as described in the next section.

3.3. Extraction of the phase relaxation kinetics for each transition and discussion

Taking into account that phase relaxation kinetics originating from different mechanisms is a product of the partial kinetics due to each mechanism, for any particular position within the GdSL EPR spectrum, the phase relaxation kinetics can be represented as

$$V(\tau, B) = \sum_n S_n V_n(\tau) = \sum_n S_n V_n^Z(\tau) V_n^o(\tau) \quad (1)$$

where we designate the t ZFS driven phase relaxation kinetics as $V_n^Z(\tau)$, and the kinetics due to other mechanisms as $V_n^o(\tau)$, $n = 0 \dots 3$, assigned to ($\langle -1/2 \rangle \leftrightarrow |1/2 \rangle$), ($\langle \pm 3/2 \rangle \leftrightarrow |\pm 1/2 \rangle$), ($\langle \pm 5/2 \rangle \leftrightarrow |\pm 3/2 \rangle$) and ($\langle \pm 7/2 \rangle \leftrightarrow |\pm 5/2 \rangle$) transitions, respectively. The weights of these transitions collected at particular magnetic fields are designated as $S_n \equiv S_n(B)$. The $S_n(B)$ ’s are calculated from the simulations shown in Fig. 4. The simulated spectrum composition shows that in the range of $|B| > 120$ mT, only the ($\langle \pm 7/2 \rangle \leftrightarrow |\pm 5/2 \rangle$) transitions contribute to the echo. Accordingly, the phase relaxation within these field intervals (*e.g.*, traces 6, 7 in Fig. 2a) is given by, $V(\tau, B^{(3)}) \equiv V_3(\tau)$, and

$$V_3^Z(\tau) = V_3(\tau) / V_3^o(\tau) \quad (2)$$

$V(\tau, B^{(2)})$, collected at any $B^{(2)}$ is:

$$V(\tau, B^{(2)}) = S_2(B^{(2)})V_2(\tau) + S_3(B^{(2)})V_3(\tau) \quad (3)$$

Since $V_3(\tau)$ is already determined, $V_2(\tau)$ related to the $(\langle \pm 5/2 | \leftrightarrow | \pm 3/2 \rangle)$ transitions is readily obtained from:

$$V_2(\tau) = (V(\tau, B^{(2)}) - S_3(B^{(2)})V_3(\tau)) / S_2(B^{(2)}) \quad (4)$$

and

$$V_2^Z(\tau) = V_2(\tau) / V_2^o(\tau) \quad (4')$$

Finally, $V(\tau, B^{(1)})$ measured at any $B^{(1)}$ is:

$$V(\tau, B^{(1)}) = S_1(B^{(1)})V_1(\tau) + S_2(B^{(1)})V_2(\tau) + S_3(B^{(1)})V_3(\tau) \quad (5)$$

$$V_1(\tau) = (V(\tau, B^{(1)}) - S_3(B^{(1)})V_3(\tau) - S_2(B^{(1)})V_2(\tau)) / S_1(B^{(1)}) \quad (5')$$

and

$$V_1^Z(\tau) = V_1(\tau) / V_1^o(\tau) \quad (5'')$$

The ZFS has a very small effect on the energies of the $-1/2$, $1/2$ states, which is evident from the line shape of the $\langle -1/2 | \leftrightarrow | 1/2 \rangle$ transition. While for all other transitions the characteristic line width is proportional to D_Z , at W-band the $\langle -1/2 | \leftrightarrow | 1/2 \rangle$ transition it is compressed by about $B/(10 \cdot D_Z) \approx 15$ times [36,37]. By definition, the phase relaxation of the echo signal is proportional to the magnitude of energy state fluctuations. Intuitively, it seems reasonable to assume that energy fluctuations of the $-1/2$, $1/2$ states due to t ZFS should be scaled down (as compared with other transitions) by the same order of magnitude as the line width of this transition.

These intuitive arguments are supported by prevailing knowledge that in a liquid phase, even under the most unfavorable conditions, the relaxation rate of $\langle -1/2 | \rightarrow | 1/2 \rangle$ transition is at least an order of magnitude lower than that of $\langle 5/2 | \leftrightarrow | 7/2 \rangle$ transitions [31–33]. These arguments, which will be further supported in our discussion, were used for the following data processing, where we assumed that the phase relaxation of $-1/2$, $1/2$ spin states is similar to that of $S = 1/2$ [42], that is to say it is driven by stochastic modulations of electron-nuclear interaction (n SD) plus spin-lattice relaxation.

To extract $V_n^Z(\tau)$, from the experimental data, one needs to evaluate $V_n^o(\tau)$. For the purposes of this work, the knowledge of the exact mechanisms causing $V_n^o(\tau)$ is not essential, but it is important to know how $V_n^o(\tau)$ varies with m_s or n . Under our experimental conditions, the

main mechanism contributing to $V_n^o(\tau)$ is temperature independent n SD [43]. There could be some minor admixture of phase relaxation induced by temperature dependent spin lattice interactions starting from 18 K, but not at 10 K, as it is shown in Section 3.5. We first assume that $V_n^o(\tau)$ is transition independent and is given by $V_0^o(\tau)$. The latter can be initially taken from the experimental phase relaxation data acquired at $B^{(0)}$, since about 85% of the EPR signal intensity at this field due to the $\langle -\frac{1}{2} | \leftrightarrow | \frac{1}{2} \rangle$ transition. Under this assumption, according to Eq. (2):

$$\ln V_3^Z(\tau) = \ln V_3(\tau) - \ln V_0^o(\tau) \quad (6)$$

where $\ln V_3(\tau)$ and $\ln V_0^o(\tau)$ are traces (6,7) and (1) in Fig. 2, respectively. The result of this data decomposition is shown in Fig. 5. Due to high SNR of $V_0^o(\tau)$ the SNR for $V_3^Z(\tau)$ remains about the same as $V_3(\tau)$. To avoid unnecessary noise amplification in the following steps of kinetics processing, we used a cubic spline to fit a noiseless (or close to a noiseless) curve through the experimental $V_3(\tau)$ curve. This curve was used in the next step to extract $V_2^Z(\tau)$. The same procedure was then used to fit the curve through $V_2(\tau)$ and the latter was finally used to extract $V_1^Z(\tau)$. The spline fitting procedure is incorporated in our standard processing software, SpecLab [41]. (SpecLab can be downloaded, free of charge, from http://www.cbc.Arizona.edu/facilities/epr_facility_software)

Next we attempt to explain the major experimental observations in terms of the specifics of t ZFS. As is evident from Fig. 5, the decay due to this phase relaxation process increases from $n = 1$ to $n = 3$ and the ratio of $\ln V_3^Z(\tau) / \ln V_1^Z(\tau)$ at $\tau = 12 \mu\text{s}$ is somewhere between 2.8 and 3.0. Additional analysis shows that kinetics 2 and 3 can be transformed into each other, within the limits of experimental accuracy, by rescaling. The dependence of these kinetics on τ is close to $\tau^{1/2}$, as shown in Fig. 5. Close analysis of kinetics 1 shows that its shape slightly deviates from that of kinetics 2 and 3 and its dependence on τ is somewhere between $\tau^{1/2}$ and τ . The deviation is not very significant. At least one factor, such as the accumulation of error while progressing from $V_3^Z(\tau)$ to $V_1^Z(\tau)$ in the data analysis, could cause this effect. A sedulous conclusion regarding the shape of the kinetics should be based, therefore, on the shape of $V_3^Z(\tau)$, and so in comparing relaxation efficiency, we will refer to decays at the end of the time base. We also evaluated $V_n^Z(\tau)$ using other kinetics collected at field locations different from those presented in Fig 2. The results were very close to those presented in Fig. 5.

In liquids (indexed as l in following equations), the t ZFS driven kinetics of phase relaxation are exponential [31–33],

$$\ln V_n^{Z,l}(t) = -W_n t \quad (7)$$

where W_n is the relaxation rate. Evidently, the time dependence given by Eq. (7) and that observed in our experiment are different ($-\ln V(t) \propto t$ in liquids vs $\propto \tau^n$ in glasses) but this comes as no surprise. In glasses, the ZFS tensor parameters of GdSL are broadly distributed and so it is reasonable to expect that parameters of t ZFS are distributed as well. Such a

distribution should subsequently lead to a distribution of phase relaxation rates. In this framework of reasoning, the resulting value for kinetics is the sum of individual kinetics, that is

$$f(\tau) = \sum g^{(i)} \exp(-2W^{(i)}\tau) \rightarrow \int f(W) \exp(-2W\tau) dW \quad (8)$$

where $g^{(i)}$ and $W^{(i)}$ are the weight and relaxation rate of a given configuration i and $f(W)$ is the rate distribution function. Evidently, the distribution of relaxation rates transforms mono-exponential kinetics into a more complex function, such as $f(\tau) \propto \exp(-a\tau^\eta)$, $\eta \neq 1$. For instance, $f(\tau) \propto \exp(-a\sqrt{\tau})$ ($\eta \equiv 1/2$) is realized for a ‘Margenau distribution’ of W [28].

To proceed further, in the following analyses, we assume that the basic pattern of the phase relaxation of Gd^{3+} in glasses remains the same as in liquids [31–33] but that the relaxation rates are distributed. Under such an assumption, $\ln V_n^{Z,l}(t)$ (Eq. (7)) transforms into

$$\ln V_n^Z(\tau) \propto -c_n \tau^\eta \quad (9)$$

The c_n in Eq. (9) is

$$c_n = (\alpha_n \Delta'^2 \tau_c^\#)^\eta \quad (10)$$

where $\tau_c^\#$ is the characteristic correlation time of the t ZFS stochastic modulation, and Δ' is the t ZFS characteristic value. In liquids, Δ' is defined as $(\Delta')^2 = \frac{2}{3}D^2 + 2E^2$ where D and E are commonly, but not always, chosen to be equal to the parameters of the static ZFS tensors. The magnitude of the coefficient α_n depends on $\omega_0 \tau_c^\#$, the product of operational frequency, ω_0 , and correlation time, $\tau_c^\#$. For $\omega_0 \tau_c^\# \ll 1$, for $n = 1, 2$ and 3 , respectively, the α_n ’s are about 95, 60, and 32 while for $\omega_0 \tau_c^\# \gg 1$ they are (54/5), (24/5), (6/5). (For $\omega_0 \tau_c^\# \ll 1$, the rates for $\langle 5/2 | \leftrightarrow | 7/2 \rangle$ and $\langle -5/2 | \leftrightarrow | -7/2 \rangle$ transitions are slightly different, but this is not relevant to our discussion.) [31–33] In accordance with our assumptions, in glasses we could expect the ratio of the c_n ’s (see Eq. (10)) to be the same as the ratio of α_n , that is $1:2^\eta:3^\eta$ for $\omega_0 \tau_c^\# \ll 1$ and $1:4^\eta:9^\eta$ for the reverse situation. Since the t -dependence of the logarithms in the experimental kinetics is close to $\sqrt{\tau}$, in order to compare the experimental data with the estimates above, we set $\eta = 0.5$ and get ratios for c_n ’s as $1:1.4:1.7$ for $\omega_0 \tau_c^\# \ll 1$ and $1:2:3$ for $\omega_0 \tau_c^\# \gg 1$. The latter is closer to the experimental ratios (1:1.8:2.9) than the former. Nevertheless, to be on the safe side in assigning $\omega_0 \tau_c^\#$, we attempted to estimate the correlation times independently.

The evaluation of product $\alpha_n (\Delta')^2 \tau_c^\#$ is rather straightforward. To achieve this, one can simply use one of the kinetics presented in Fig. 5, for instance, kinetics 2. The decay of kinetics 2 at a time base of $\tau \sim 12 \mu\text{s}$ is about e^{-1} , therefore $\alpha_2 (\Delta')^2 \tau_c^\# \sim 10^5 \text{s}^{-1}$. In the worst case scenario of $\omega_0 \tau_c^\# \ll 1$, $\alpha_2 = 60$ [32]. As an estimate, we’ll simply set $\alpha_2 = 10^2$, which

gives $(\Delta')^2 \tau_c^\# \sim 10^3 \text{ s}^{-1}$. Evidently, the $\tau_c^\#$ estimate requires an independent estimate of Δ' . Despite the vast number of publications on the evaluation of static and transient ZFS parameters (see, e.g., [44–47] and refs. within), there are no compelling procedures on how to address this problem. The most relevant approach was used by Dzuba [21] to separate the angle of ‘small angle libration’ and the correlation time of molecular reorientation in the low temperature phase relaxation of a nitroxide probe. Based on a previous investigation [48], it was explicitly shown that while in pulsed EPR, the spin motion results in phase relaxation for anisotropic interaction, in continuous wave EPR, it causes an EPR line shift from the ultimate static position. The magnitude of this shift is about the same magnitude as the transient anisotropic interaction. Such an approach cannot be directly applied for estimating Gd^{+3} t ZFS in glasses where the interaction with eight ligands and the overlap of lines from different transitions obscure any possible effects of anisotropic interactions. In single crystals doped with Gd^{+3} ions, however, individual lines from various transitions of Gd^{+3} are resolved and their positions are affected by t ZFS. These t ZFS driven shifts of line positions are consequently incorporated into the magnitude of measured ZFS parameters. The apparent effects of t ZFS are usually revealed through a temperature dependence of the ZFS parameters (see, e.g., [49–51]). Commonly, below 30–50 K, the variations of ZFS parameters are not detectable within the limits of accuracy for the measurements. The latter, therefore, can be taken as a plausible estimate for t ZFS. General consensus is that the ZFS parameter, D_Z , is measured with relative accuracy between $\pm 1 \cdot 10^{-3}$ and $\pm 3 \cdot 10^{-4}$. [49–51] Such an accuracy sets the magnitude of Δ' somewhere around 1 MHz for Gd -DOTA and, as $(\Delta')^2 \tau_c^\# \sim 10^3 \text{ s}^{-1}$, the value of $\tau_c^\#$ could reach about 10^{-9} s. As a result, for W-band measurements, $\omega_0 \tau_c^\#$ substantially exceeds unity, contrary to the worst case scenario assumed above that $\omega_0 \tau_c^\# \ll 1$. This narrows the limits of t ZFS driven relaxation rate ratios for various transitions to be close to (1:1.8:2.9) and simplifies the expression for the t ZFS driven rate of the $-1/2 \rightarrow 1/2$ transition, α_0^η , relative to the rate of any other transition α_n^η . For instance, the ratio of $(\alpha_0/\alpha_3)^\eta$ estimated using the relaxation matrix presented in [31,32] is about $(0.55/(\omega_0 \tau_c^\#))^2$. For $\eta = 0.5$ this ratio is about $0.75/(\omega_0 \tau_c^\#)$. As a result, at W-band the t ZFS driven relaxation of the $-1/2 \rightarrow 1/2$ transition should only be about 1% of that for $\pm 5/2 \leftrightarrow \pm 7/2$ transitions (obtained for $\tau_c^\# \sim 10^{-9}$ s), less than $\exp(-0.03)$ in the experimental time base, as evaluated from the data presented in Fig. 2. Such a magnitude of t ZFS driven decay is substantially lower than the experimental one for the $-1/2 \rightarrow 1/2$ transition. This supports our qualitative arguments that n SD is the prevailing mechanism of phase relaxation of the $-1/2 \rightarrow 1/2$ transition.

It is easy to demonstrate that Δ' in the MHz range can be readily obtained due to very small ligand displacements or any other motions, like ‘small angle libration’ or wobbling. Indeed, in accordance with Newman et al. [39,40], the dependence of Δ' on the displacement, δr , between the central ion and a ligand is

$$\Delta' = \zeta \frac{\delta r}{R_0} D_Z \quad (11)$$

where R_0 is the nominal value of the Gd^{3+} -ligand distance and ζ is around 2–3. From Eq. (11) it follows that $\delta r \sim 10^{-2} \text{ \AA}$ is sufficient to set $\delta \nu$ in the MHz range. An even smaller value would be enough if the displacement of all 7–9 ligands of GdSL is taken into account. A simple evaluation of the displacements shows that δr in the range of a few hundredths of an angstrom is quite realistic [52]. Likewise, in the same framework of the superposition model, the ZFS parameters of an individual ligand have an intrinsic angular dependence on the Gd^{3+} -ligand direction in the local molecular coordinate frame and that small deviations from this direction can readily result in $\sim 1 \text{ MHz}$ of variation for D_Z . An accurate estimate of this deviation is complicated due to the presence of numerous ligands, but in accordance with [39,40], it should be less than $\delta \nu / D_Z$. This means that small librations in the range of tenths of a degree can generate $\delta \nu$ in the MHz range.

The magnitude of $\tau_c^\#$ remains, however, an unresolved issue. The discussion above places $\tau_c^\#$ in the range of 10^{-8} – 10^{-9} s , which is rather slow for lattice vibrations (10^{-11} – 10^{-14} s). The low frequency vibrational modes in amorphous ice were observed experimentally and explained theoretically (see Ref. [53] and refs. within for details). The maximum density of states (DOS) distribution for these modes, however, was found to be around 10 – 20 cm^{-1} , (ps range of correlation times), although the “tail” of the distribution is spread over substantially lower frequencies. Apparently, only the very low frequency part of the vibrational modes is efficient in inducing phase relaxation, but a clear physical picture of the development of these modes is yet to be understood and the DOS of these modes is yet to be evaluated. Although similar observations of slow ‘small angle librations’ of nitroxide probes were reported in [21], a concise explanation of how such slow motions develop still remains elusive.

3.4. Phase relaxation – temperature dependence

Typically in research that is directed at understanding the origin of correlation times, the temperature dependence of the relaxation kinetics is investigated. We carried out phase relaxation measurements in the range of 6.6–18 K (see Fig. 6). At lower temperatures, long term temperature stability is challenging for the existing cryogenic equipment; at temperatures greater than 18 K, the phase relaxation induced by spin lattice relaxation becomes quite efficient (see Section 3.5). Already at 18 K, there is a substantial increase in phase relaxation (see Fig. 6a). Accordingly, off center measurements were collected in the 6.6–14 K range.

As evident from Fig. 6a, in the 6.6–14 K range, the variation of the kinetics collected in the center of the EPR spectrum with temperature is subtle, as is also for the kinetics collected at the fringes of the EPR spectrum, $B_0 = \pm 140 \text{ mT}$, presented in Fig 6b. Because the kinetics collected at $B_0 = 140 \text{ mT}$ were very close to those at $B_0 = -140 \text{ mT}$, the latter are not shown in Fig. 6b. Q-band Gd^{3+} phase relaxation measurements for $B_0 = 0$, gave a similar subtle dependence [15]. Since $\ln V_3^Z(\tau)$ is the difference between the respective pairs presented in Fig. 6a and b, it is evident that $\ln V_3^Z(\tau)$ vary only by about 20–30% by the end of time interval, while the temperature varies by more than twofold. Apparently, the temperature dependence of the $\delta \nu$ driven phase relaxation in this temperature range is quite moderate.

The exact reasons for such a weak temperature dependence are not clear but it could be an indication of the simple fact that τ_c' and $\tau_c^\#$ are inversely correlated. In future research, it would be interesting to decrease the temperature further to the point where the ligand fluctuations becomes frozen in our observation time scale. This may provide us with a more or less independent evaluation of correlation times and a better understanding of the magnitude and nature of t_{ZFS} .

3.5. Spin lattice relaxation

Although spin lattice relaxation is not the focus of this work, we measured it in order to determine its role in the phase relaxation processes. The temperature dependence of the inversion recovery curves of 20 μ M Gd-DOTA is shown in Fig. 7 in the form of

$\varphi(t) \equiv \ln \left(\left(1 - \frac{V(t)}{V_0} \right) / 2 \right)$ as a function of t , where V_0 is the amplitude of the primary echo when the inversion pulse is switched off.

As an example, the inversion recovery kinetics, $\varphi(t)$, collected at 10 K at various positions within the EPR spectrum are presented in Fig. S2. Three other sets were also collected at 6.6, 14 and 18 K, and exhibit a similar behavior. The field dependent measurements show that (i) the inversion recovery kinetics are not exponential and (ii) they are practically independent of field position (or on the particular transitions), in contrast to phase relaxation. Measurements carried out using saturation recovery (not shown) showed the same behavior. In principle, the data can be fitted to a bi-exponential decay with slow and fast components. We chose to analyze the data in terms of a broad distribution of relaxation times because it makes it easier to generalize the behavior. Next, we derived a general empiric dependence of the spin lattice relaxation on temperature in the range of 6.6–18 K. The kinetics can be fitted to a stretch exponential $\varphi(t) \propto \alpha t^\varepsilon$, $0.6 < \varepsilon < 0.7$. The implications of this behavior will be discussed later. The distribution of spin lattice relaxation rates, W_{1L} , leads to the following longitudinal magnetization, $M_z(t)$:

$$M_z(t) \propto \int f(W_{1L}) \exp(-2W_{1L}t) dW_{1L} \propto \exp(-\alpha t^\varepsilon), \quad (12)$$

where $f(W_{1L})$ is the distribution function of individual spin lattice relaxation rates and $\alpha \propto (W_{1L}^*)^\varepsilon$; W_{1L}^* is a characteristic parameter (or set of parameters) of $f(W_{1L})$. One of the possible reasons for the W_{1L} distribution is presented below.

Fig. 7a and b show that whereas the temperature affects the rates of the decay, the shape of $\varphi(t)$ seems to be invariant. This indicates that ε does not depend on temperature in the range of temperatures studied. Such an observation allows for the evaluation of an apparent temperature dependence of a characteristic rate of spin lattice relaxation, $\alpha(T)$ under the standard assumption that $\alpha(T) \propto T^p$. Next we have to evaluate p . Defining $t^* \equiv t^*(T)$ for which $\varphi(t^*(T))$ is equal at various temperatures (see Fig. 7a) and using Eq. (12) one obtains:

$$\rho(\ln T) \propto (\ln t^*) \quad (13)$$

Accordingly ρ is proportional to the slope of the dependence of $\ln(t^*)$ on $\ln T$ and the data presented in Fig. 8 yield $\rho \approx 1-1.2$ for $0.6 < \varepsilon < 0.7$.

To find the intrinsic temperature dependence of spin lattice relaxation rates, we assume that the temperature dependence of the individual longitudinal relaxation rate, W_{1L} , is the same for each molecule. Under such an assumption, $W_{1L} \propto T^{\rho/\varepsilon}$ and, $(\rho/\varepsilon) \approx 1.7$ (see Eq. (13) and comments for this Eq.) The intrinsic temperature dependence of the spin lattice relaxation rates can therefore be represented as $\propto T^{1.7}$. The spin-lattice relaxation of Gd^{3+} ions in various matrices was the subject of a number of investigations. The most thorough investigation was performed by Thorp et al. [54] for Gd^{3+} ions in $CaWO_4$ single crystals in the 1.5–30 K temperature range. It was shown that in the range 1.5–8 K, the ‘direct’ process of spin lattice relaxation ($W_L \propto T$) prevails, while for 10–30 K, the major relaxation mechanism is a ‘Raman process’ for which $W_L \propto T^3$. The low power of the temperature dependence of the Raman process, as opposed to the much higher power expected [36] was attributed to the high defect concentration.

The temperature dependence of $W_{1L} \propto T^{1.7}$ observed in our experiments is somewhere between T^3 and T , which is probably of comparatively equal importance to the two above mentioned relaxation processes for the temperature range used in our experiments. Interestingly, although it may be purely coincidental due to the quite different nature of the host matrices, the temperature range of 6.6–18 K in which our measurements were performed coincides with that where the transformation for T to T^3 dependence occurs [54]. It was also found that spin lattice relaxation rates are considerably anisotropic [54]. In disordered systems, such anisotropy should manifest itself as a static distribution of relaxation rates.

We note that the presence of the ZFS can be neglected when considering the spin lattice relaxation of S-ions in solids because the ZFS energy is substantially smaller than the Zeeman quantum, as was emphasized by Blume and Orbach [55]. This is consistent with our observation that that spin–lattice relaxation for GdSL is transition independent.

Finally, one more experimental feature should be mentioned. Previously, when discussing the source of phase relaxation, we mentioned that at 18 K, the spin lattice relaxation becomes an additional channel for phase relaxation, the efficiency of which is comparable with phase relaxation caused by nuclear spectral diffusion. Indeed, as is evident from the inset in Fig. 7b, at 18 K, between 1 μ s and 24–25 μ s, $\phi(t)$ (which represents a measure of the spin lattice relaxation) varies by about 0.8. A similar magnitude of additional decay is observed in the kinetics of phase relaxation at 18 K. This is apparent from the comparison of the phase relaxation kinetics collected at 6.6 (or 10 K) and 18 K, as shown in Fig. 6.

3.6. Phase relaxation-phenomenological approach

In previous sections, we gave a comprehensive analysis of an additional mechanism for the phase relaxation of Gd^{+3} . This information, together with already known effects from other mechanisms, allow us to estimate the variation of the phase relaxation along the Gd^{+3} spectrum and then use it for optimization of pulse EPR measurements on Gd^{3+} general and DEER in particular. Understanding, however, that such an approach to measurement

optimization might be too arcane for those researchers involved in solving just structural biological problems by means of pulse EPR, we offer below a simple phenomenological dependency of the phase memory time on Gd^{3+} concentration, solvent composition and temperature, which can serve as a guideline in setting the conditions for particular experiments, namely where should the pump or observe pulses be placed. Some more elaborate discussion of this can be found in [16]. In biological applications, variation of label concentration is not always possible, the concentration used above, 0.02 mM GdSL being too low, and solution composition could be far from optimal. The experiments described below were performed, therefore, for a solution composition of 0.3:0.7 v:v glycerol–water, which is often used in biological applications and the concentration was varied between 0.02 to 0.2 mM, the range typical for DEER measurements. The echo decays were measured at two field positions; the maximum of the central transition and 10 mT (280 MHz) up field. These positions are indicated in the Gd-DOTA ED-EPR spectrum, as shown in Fig. 9, and reflect a possible setup in the W-band DEER measurements for low-Q, broadband, or bi-modal resonators. From our experience with Gd^{3+} – Gd^{3+} DEER using Gd-DOTA derived spin labels this the behavior is similar for a 100 MHz offset, which is also situated outside the range of the central transition. Since the phase relaxation kinetics are, in general, non-exponential, to facilitate a convenient measure of the characteristic decay time, we chose to use τ value at which the echo decayed to 10% of its initial value, $\tau_{10\%}$ (see Fig. S3 for examples of echo decay traces). This value gives an approximate idea of the maximum evolution time available in a DEER measurement. The echo amplitude at $\tau = 0$ could not be determined because of spectrometer dead time (~ 100 ns) and was obtained by linear extrapolation.

The observations shown in Fig. 9 are summarized as follows: (i) For the concentration range 0.02–0.2 mM $\tau_{10\%}$ at the maximum of the EPR spectrum (dominated for the central transition) is concentration dependent, increasing with decreasing concentration. (ii) $\tau_{10\%}$ measured off center is consistently shorter than that measured at the central position and it is practically concentration independent. (iii) The temperature dependence of $\tau_{10\%}$ measured at the maximum of the EPR spectrum is pronounced but becomes milder at 0.02 mM, in contrast, the temperature dependence of $\tau_{10\%}$ measured off center is fairly constant at the concentration range studied. The stronger concentration dependence of $\tau_{10\%}$ at the maximum of the EPR spectrum is expected considering the dominance of the central transition and its narrower linewidth, for which spectral diffusion and instantaneous diffusion are expected to be more efficient.

While the above characteristics of $\tau_{10\%}$ are consistent with the results and analysis presented in 3.1–3.4, some features are more accentuated. A linear extrapolation based on the linear end part of the data of the 0.02 mM solution given Fig. 6a give estimated $\tau_{10\%}$ values of 25, 23, 21 and 16 μs for 6.6, 10, 14 and 18 K, respectively. These are slightly longer than those presented in Fig. 9. This indicates that the *iD* mechanism at this glass composition (7:3, vs 1:1) D_2O /glycerol- d_8 is slightly more efficient than expected for random distribution, apparently reflecting the beginning of GdSL clusterisation for the 7:3 solution. In addition, the up-field phase relaxation of GdSL at this glass composition shows a slightly more significant temperature dependence than that observed in Fig. 6, 6.6–14 K. This could be a

consequence of the longer time base where phase relaxation related to spin lattice interactions become more efficient at lower temperatures. This problem requires further investigation. Additional testing of the sensitivity of $\tau_{10\%}$ to the solvent was performed for different compositions of D₂O/DMSO-d₆. The results, given in Fig. S4, show that $\tau_{10\%}$ is highly sensitive to the solvent composition and in this particular case the longest phase relaxation was observed for 3:7 D₂O/DMSO-d₆, which suggests that this composition yield the best glass such that the Gd-DOTA is homogenously distributed and clustering is avoided. The results are compared with the obtained for 7:3 (v/v) D₂O/glycerol-d₈ glasses because of its use in DEER investigations of bio-macromolecules. The phase relaxation observed is similar to the 3:7 D₂O/DMSO-d₆. For comparison recent measurements of the phase relaxation of a GdSL in a nitroxide-Gd³⁺ labeled protein measured at 10 K and at the maximum of the EPR signal, gave $\tau_{10\%}$ values around 5 μ s at both X- and Q-band [15].

4. Conclusion

In this work, we focused on the phase relaxation of Gd³⁺ chelates in glassy matrices at cryogenic temperatures and discovered an additional, transition dependent mechanism. We suggested that this mechanism is caused by *t*ZFS, as in liquids, but with substantially scaled down and distributed *t*ZFS and correlation times. This ZFS-induced phase relaxation mechanism becomes dominant (or at least significant) when all other well-known phase relaxation mechanisms, such as *n*SD, *e*SD and *i*D, are significantly suppressed by matrix deuteration, the use of low concentration, and when the temperature is low enough to disable spin lattice interaction as a source of phase relaxation. Although most of the features of the additional mechanism are generally understood, one particular issue, the nature of low frequency lattice vibrations (or restricted librations) causing *t*ZFS, remains unclear and has to be addressed in further research. In terms of applications such as DEER measurements, this work provides a deeper understanding of the nature of phase relaxation and it allows making a sedulous choice of field position for the pumping/observation pulses necessary to achieve optimal SNR for any particular distance measurement. In the future, it will be necessary to resort to ligand deuteration in order to evaluate the contribution to spin relaxation from the protons of the chelate backbone.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jmr.2014.09.012>.

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Fig. 1.
The Gd^{3+} compound studied in this work.

Fig. 2.

(a) Selected kinetic data of Gd-DOTA phase relaxation collected for various B_0 values given with respect to the maximum for the central transition, (1) $B_0 = 0$ mT, (2,3) $B_0 = \pm 5$ mT, (4,5) $B_0 = \pm 60$ mT and (6, 7) $B_0 = \pm 140$ mT. ($\pm$) correspond to solid and dashed lines, respectively. The nominal $\pi/2$, π pulse durations were 12.5, 25 ns; mw frequency = 94.8 GHz. 'Zero' of the magnetic field corresponds to ~ 3401 mT. (b) ED-EPR of Gd-DOTA at 10 K. Experimental conditions: mw frequency = 94.9 GHz; $\pi/2 - \pi$ pulses of 30 ns and 60 ns; $\tau = 500$ ns; repetition rate 1 kHz; magnetic field sweep 0.2 mT/s; 'Zero' of magnetic field corresponds to ~ 3405 mT. Arrows show field positions at which measurements of phase relaxation were performed.

Fig. 3.

(a) Simulated (solid) and experimental (dashed) spectra of Gd-DOTA at 10 K. Due to the large difference in width and intensity of the subspectra for the $\langle -\frac{1}{2} | \leftrightarrow | \frac{1}{2} \rangle$ transition and for all of the other transitions, the spectral region of the latter is shown separately in (b). For a detailed comparison of the experimental and simulated spectra, the central part of the spectra presented in (b) has been removed. Simulations were performed using an in-house simulation program 'highspinsym' [41].

Fig. 4. Simulated sub-spectra of various transitions for Gd-DOTA at 10 K. (a) 1 – $(\langle 3/2 | \leftrightarrow | 1/2 \rangle) + (\langle -3/2 | \leftrightarrow | -1/2 \rangle)$ transitions, 2 – $(\langle 5/2 | \leftrightarrow | 3/2 \rangle) + (\langle -5/2 | \leftrightarrow | -3/2 \rangle)$ transitions and 3 – $(\langle 7/2 | \leftrightarrow | 5/2 \rangle) + (\langle -7/2 | \leftrightarrow | -5/2 \rangle)$ transitions. (b) Sub-spectrum of the $\langle -1/2 | \leftrightarrow | 1/2 \rangle$ transition.

Fig. 5.

The $V_n^Z(\tau)$ phase relaxation kinetics due to i ZFS for transitions, (1) $\langle \pm 3/2 | \leftrightarrow | \pm 1/2 \rangle$, (2) $\langle \pm 5/2 | \leftrightarrow | \pm 3/2 \rangle$ and (3) $\langle \pm 7/2 | \leftrightarrow | \pm 5/2 \rangle$ at 10 K, derived under the assumption that the phase relaxation due to spectral diffusion, $V_n^o(\tau)$, is independent of m_s . $\tau^{1/2}$ The dashed line 4, is the same as (3) but plotted against $\tau^{1/2}$.

Fig. 6.

(a) Kinetics of phase relaxation collected at different temperatures for $B_0 = 0$ (center of signal). Curves labeled 1, 2, 3, and 4 correspond to 18, 14, 10 and 6.6 K respectively. Inset: phase relaxation kinetics collected for $B_0 = 0$, (zero magnitude of field is assigned to center of signal) and 10 K, before (dash-dots, curve 3 of picture body) and after (solid line) the correction for admixture of transitions other than $\langle -1/2 | \leftrightarrow | 1/2 \rangle$. (See SI for details). (b) Phase relaxation kinetics collected for $B_0 = -140$ mT at three temperatures 6.6 K, 10 K and 14 K.

Fig. 7.

(a) Inversion recovery kinetics collected at (1) 6.6 K, (2) 10 K, (3) 14 K and (4) 18 K at the maximum of the EPR spectrum. The horizontal dashed line indicates an isodecay value of $\phi(t)$ at temperature-dependent t^* values (see text). (b) Demonstration of similarity of $\phi(t)$ for 6.6 K (black line) and 18 K (red line). To match these two, the 6.6 K kinetics was compressed 5.25 times. Also, to account for the different time scale, the black curve was shifted by $-5 \mu\text{s}$ relative to the red curve. The inset shows the initial decay (shadowed area) during the first 25 μs at 18 K. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 8. Dependence of $\ln(t^*(T))$ on $\ln(T)$. The dashed line was drawn through the experimental points by means of the least square method.

Fig. 9. Dependence of $\tau_{10\%}$ on concentration and temperature for Gd-DOTA in D_2O : glycerol- d_8 (7/3 v:v). In (a), the measurements are performed on the central transition and in (b), the measurements are performed at $\nu = 280$ MHz. The inset shows part of the Gd-DOTA spectrum with the arrows indicating the magnetic fields at which measurements were carried out.