Appl. Magn. Reson. 28, 231–238 (2005)

**Applied Magnetic Resonance**



# Multifrequency EPR of Four Triarylmethyl Radicals

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Received August 16, 2004

**Abstract.** Continuous-wave spectra at W-band of four triarylmethyl (trityl) radicals at 100 K in 1:1 water–glycerol exhibit rhombic electron paramagnetic resonance spectra. The rigid-lattice line widths at W-band are only 3 to 5 times larger than at X-band or S-band, and fluid-solution line widths are much narrower than those for rigid lattice, which indicates that unresolved anisotropic nuclear hyperfine couplings make significant contributions to the rigid-lattice line widths. Spin-flip lines are observed in glassy-solution spectra at X-band and S-band, but not at W-band or 250 MHz. At 100 K $T_m$ is dominated by spin diffusion of solvent protons and is independent of microwave frequency. Between about 130 and 170 K, $1/T_m$ for trityl-$CH_3$ is enhanced by rotation of the methyl groups at a rate comparable to inequivalences in the hyperfine interaction. Motional averaging of anisotropic interactions enhances spin echo dephasing between about 200 and 300 K. The temperature dependence of $1/T_1$ is similar for the four radicals and is consistent with assignment of the Raman process and a local mode as the dominant relaxation processes. The similarity in $T_1$ values at W-band and X-band supports this assignment.

## 1 Introduction

Triarylmethyl (trityl) radicals have been prepared in which nuclear hyperfine coupling is very small and X-band peak-to-peak line widths in aqueous room temperature solutions are 23 to 80 mG [1]. The narrow lines in the electron paramagnetic resonance (EPR) spectra of these species are very sensitive to broadening by collisions with $O_2$ and show promise for in vivo oximetry [1]. To optimize design of the trityls and to select parameters for EPR experiments that utilize these radicals, it is important to understand the spectra and the factors that contribute to the electron spin relaxation rates. A preliminary report of trityl $g$-value anisotropy was given by Kamlowski et al. [2].

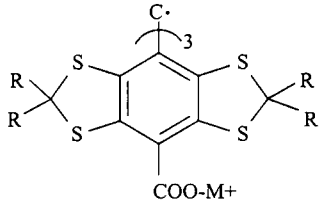
In this study continuous-wave (CW) spectra in glassy water–glycerol were compared at W-band (about 93.89 GHz), X-band (about 9.23 GHz), S-band (about 2.78 GHz) and 250 MHz to determine $g$-anisotropy, which can contribute to spin–lattice relaxation and to confirm the assignment of spin-flip lines.

The temperature dependence of spin echo dephasing and spin–lattice relaxation was measured at X-band to determine the mechanisms of these processes. Comparison of W-band and X-band relaxation rates was made at 100 K.

## 2 Materials and Methods

The preparation of the trityl radicals used in this study (Fig. 1) is described in Ardenkjaer-Larsen et al. [1]. On the basis of the results of Ardenkjaer-Larsen et al. [1] a concentration of 0.2 mM was selected as a workable compromise between minimizing intermolecular interactions and maintaining adequate signal-to-noise ratio. To ensure low-temperature glass formation, solutions were prepared in 1:1 (v:v) water–glycerol. Samples for W-band spectroscopy were contained in 0.9 mm outer diameter (OD) quartz tubes and were not deoxygenated. Samples for X-band (about 9.2 GHz) and S-band (about 2.78 GHz) spectroscopy were contained in 4 mm OD quartz tubes and deoxygenated by repeated freeze-pump-thaw cycles prior to flame sealing. Samples of trityl-$CD_3$ for 250 MHz spectroscopy were contained in 10 mm OD pyrex tubes and deoxygenated by bubbling with $N_2$ gas prior to flame sealing.

W-band data were obtained on a Bruker E680-PU with an E600-1021H cylindrical resonator. X-band CW spectra were obtained on a Varian E9 with a $TE_{102}$ rectangular resonator or on a Bruker E580 with a split-ring resonator. $T_1$ at X-band was measured at room temperature by long-pump saturation recovery on a locally constructed spectrometer [3]. Inversion recovery measurements of $T_1$ and spin echo measurements of $T_m$ as a function of temperature at X-band were measured on a locally constructed spectrometer with a modified over-coupled $TE_{102}$ rectangular resonator [4]. S-band CW spectra were obtained on a locally constructed spectrometer with a cross-loop resonator [5–7]. The 250 MHz CW spectra were obtained on a locally constructed spectrometer with a loop-gap resonator [8].




| | |
|---|---|
| R = $CD_3$ | trityl-$CD_3$ |
| R = $CH_3$ | trityl-$CH_3$ |
| R = $CH_2CH_2OH$ | OX63 |
| R = $CH_2OCH_2CH_2OH$ | OX31 |

**Fig. 1.** Structures of the four trityl radicals studied.

Spin echo dephasing time constants $T_m$ were calculated by least-square fitting of Eq. (1) to the two-pulse spin echo decays.

$$Y(\tau) = Y(0)\exp(-(2\tau/T_m)^x), \quad (1)$$

where $Y(\tau)$ is the echo intensity when the time between pulses is $\tau$ and $x$ is an adjustable parameter that is characteristic of the dephasing mechanism [9]. The time constant is denoted as $T_m$ to encompass all processes that contribute to the dephasing [10]. Spin–lattice relaxation time constants $T_1$ were obtained by fitting a single exponential to the experimental inversion recovery or saturation recovery curves.

## 3 Results and Discussion

### *3.1 CW Line Shapes and g-Values*

The W-band spectrum of trityl-$CD_3$ in 1:1 water–glycerol at 100 K (Fig. 2a) exhibits partially resolved *g*-anisotropy. Simulation of the spectrum required three inequivalent *g*-values (Table 1), although the three turning points are not resolved. Similar anisotropy was observed in the W-band spectra for trityl-$CH_3$ and OX31, but anisotropy was smaller for OX63. Although *g*-anisotropy was not resolved

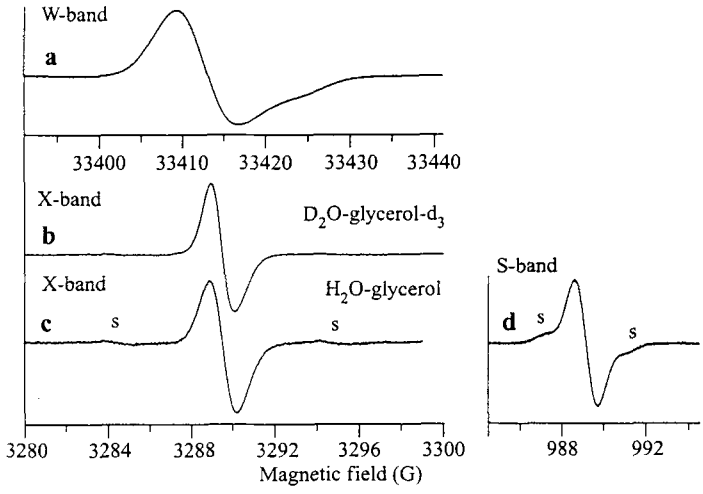



**Fig. 2.** CW spectra of trityl-$CD_3$: **a** 50 G scan at 93.65 GHz and 100 K in 1:1 water–glycerol, **b** 20 G scan at 9.227 GHz and 100 K in 1:1 $D_2O$–glycerol-$d_3$, **c** 20 G scan at 9.227 GHz and 70 K in 1:1 water–glycerol, **d** 10 G scan at 2.777 GHz and 40 K in 1:1 water–glycerol. At temperatures between 40 and 100 K the CW spectra are independent of temperature. The lines marked as 's' are assigned to spin-flip transitions.

**Table 1.** EPR parameters for four trityl radicals in 1:1 water–glycerol at 100 K.

| Parameter | Band | Value for radicals | | | |
|---|---|---|---|---|---|
| | | Trityl-$CH_3$ | Trityl-$CD_3$ | OX63 | OX31 |
| $g$-Values[a] | | 2.0030 | 2.0030 | 2.0031 | 2.0029 |
| | | 2.0027 | 2.0027 | 2.0027 | 2.0027 |
| | | 2.0021 | 2.0021 | 2.0022 | 2.0022 |
| Line widths[b] (G) | W | 4.5 | 4.5 | 5.5 | 4.0 |
| | | 4.0 | 4.0 | 4.5 | 6.5 |
| | | 8.0 | 8.0 | 6.5 | 6.5 |
| | X | 1.2 | 1.0 | 1.4 | 1.2 |
| | | 1.3 | 1.0 | 1.5 | 1.4 |
| | | 1.6 | 1.5 | 1.8 | 1.7 |
| $T_1$ (μs) | W[c] | 838 | 756 | 979 | 1042 |
| | | 808 | 678 | 946 | 979 |
| | X[d] | 1060 | 955 | 1200 | 1360 |
| $T_2$ (ESE) (μs) | W[e] | 3.1 (1.8) | 4.5 (2.2) | 4.5 (2.3) | 4.4 (2.3) |
| | X[f] | 3.9 (2.1) | 4.8 (2.4) | 5.1 (2.6) | 5.0 (2.6) |

[a] Determined by simulation of W-band spectra at 100 K. Uncertainties are ±0.0001.
[b] Peak-to-peak line widths for Gaussian first derivatives, listed in the same order as the $g$-values. Uncertainties are ±0.1 G.
[c] Pulse lengths were 60, 30 and 60 ns and 120, 30 and 60 ns, respectively. Uncertainties in $T_1$ are about ±5%.
[d] Pulse lengths were 40, 20 and 40 ns. $T_1$ at 100 K interpolated between points recorded as a function of temperature and shown in Fig. 2. Uncertainties are ±5%.
[e] Pulse lengths were 30 and 60 ns. Similar values were obtained for 60 and 120 ns pulses. The exponent, $x$, from Eq. (1) is given in parentheses. Uncertainties are about 10%.
[f] Pulse lengths were 40 and 80 ns. The exponent, $x$, from Eq. (1) is given in parentheses. Uncertainties are about 10%.

in the X-band spectra (Fig. 2c), the line shapes are consistent with the $g$-anisotropy determined from the W-band spectra. The rhombicity of the $g$-matrix indicates that the trityls in frozen solution are distorted from 3-fold symmetry. The peak-to-peak line widths of the Gaussian lines that were used to simulate the spectra were in the range of 4 to 8 G at W-band and 1.0 to 1.8 G at X-band (Table 1). The decrease in line widths by a factor of 3 to 5 from W-band to X-band is much less than the decrease in microwave frequency/field by a factor of 10, which indicates that $g$-anisotropy and $g$-strain account for only a part of the line widths. There is little change in line widths between X-band, S-band, and 250 MHz, so these rigid lattice line widths are determined largely by unresolved hyperfine interactions. Since the X-band, S-band, and 250 MHz rigid-lattice line widths are much greater than the fluid-solution line widths, which are less than 100 mG [11], these rigid-lattice line widths must arise largely from anisotropic hyperfine interaction. The peak-to-peak line width for the X-band signal from trityl-$CD_3$ in 1:1 $D_2O$–glycerol-$d_3$ at 100 K (Fig. 2b) is about 1.1 G, which is significantly narrower than the value of 1.5 G observed in normal iso-

tope abundance solvent and indicates that exchangeable protons make significant contributions to the line widths. One possibility is that the carboxylate groups are protonated and that dipolar coupling to these protons contributes to the rigid-lattice line widths. Solvent protons also may contribute.

In addition to the central line in the X-band spectrum of trityl-$CD_3$, there is a pair of satellite lines separated from the central line by about 5 G (Fig. 2c). The intensity of each satellite line is about 2.5% of that for the central line. These satellite lines also were observed in X-band spectra of the three other trityls at 100 K. In the S-band spectrum of trityl-$CD_3$ (Fig. 2d) the spacing between the central line and each satellite is reduced to about 1.3 G and the relative intensity is increased to about 18%. The decrease in the energy separation and increase in intensity with decreasing frequency is the behavior predicted for spin-flip transitions [12, 13]. These transitions are not detectable in CW spectra of trityl-$CD_3$ at 250 MHz, which is consistent with calculations that show the energy separation at 250 MHz is too small to be resolved from the central line. The satellite lines also are not observed at W-band, where calculations predict very low intensities. The intensities of the spin-flip lines depend upon the number of protons contributing and the distance from the unpaired electron. In trityl-$CD_3$ there are no protons (except perhaps the carboxylate protons) in the molecule and the structure is relatively compact so the distance of closest approach of solvent protons is about 0.7 nm [1, 9]. The observed intensities of the spin-flip lines [12, 13] are consistent with these dimensions. The intensities of these satellite lines in the X-band spectra are reduced when the exchangeable solvent protons are deuterated (Fig. 2b), which also is consistent with assignment to spin-flip lines.

### *3.2 Electron Spin Relaxation Times*

The temperature dependence of X-band values of $T_m$ are shown in Fig. 3. At 100 K, the values of $T_m$ for the four radicals are between 3 and 5 μs and are similar at X-band and W-band (Table 1). These values of $T_m$ and values of $x$ (Eq. (1)) greater than 2 are consistent with dephasing dominated by nuclear spin diffusion in a solvent that does not contain methyl groups [9]. The large radii of the trityl molecules makes the distance of closest approach to solvent protons relatively large, and so $T_m$ is longer than for nitroxyl radicals in the same solvent. For OX63 and OX31 there is little temperature dependence of $T_m$ between 77 and about 180 K, as expected for nuclear spin diffusion. For trityl-$CH_3$ in this temperature interval there is a distinctive enhancement in $1/T_m$ with maximum rate at about 160 K. This enhancement is attributed to rotation of the trityl methyl groups at a rate comparable to inequivalences in electron–proton couplings, analogous to what has been observed in nitroxyl radicals and in Cr(V) complexes [14]. A much smaller variation in $1/T_m$ in the same temperature interval for trityl-$CD_3$ may be the analogous effect, but for the much smaller electron–deuteron couplings. For trityl-$CD_3$ in $D_2O$–glycerol-$d_8$, values of $T_m$ at temperatures below about 130 K were longer than in $H_2O$–glycerol and were strongly

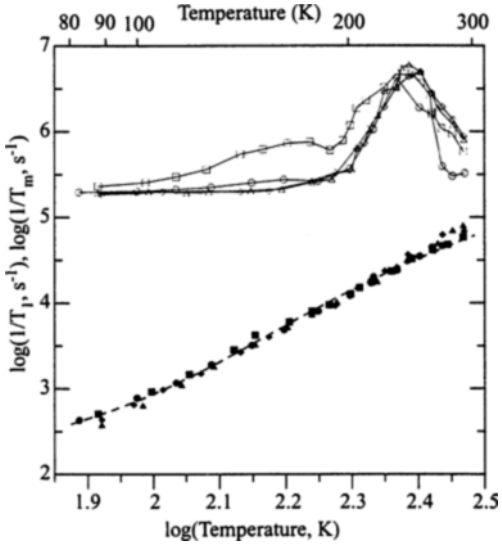



**Fig. 3.** Temperature dependence of electron spin relaxation times at X-band for 0.2 mM solutions in 1:1 water–glycerol. $T_1$ measured by inversion recovery for trityl-$CD_3$ (●), trityl-$CH_3$ (■), OX31 (◆) and OX63 (▲). The dashed line is the fit to the data for trityl-$CD_3$ including data at lower temperatures than are shown in this plot [11]. $T_m$ measured by two-pulse spin echo for trityl-$CD_3$ (○), trityl-$CH_3$ (□), OX31 (◇), and OX63 (△). The solid lines connect the data points.

influenced by instantaneous diffusion, which confirms that in proton-containing solvents, nuclear spin diffusion dominates the dephasing. Above 130 K, values of $T_m$ for deuterated solvents were the same, within experimental error, as the values for proton-containing solvents, which confirms that at these higher temperatures, nuclear spin diffusion is not the dominant contribution to $T_m$.

Between about 200 and 300 K there is a dramatic increase in $1/T_m$ for each of the trityls. This temperature range encompasses the softening point of the glass so the enhanced dephasing arises from tumbling at a rate comparable to the anisotropies in $g$- and $A$-values. At room temperature the dephasing rates reflect residual incomplete motional averaging and contributions from $1/T_1$. Since $T_1$ for trityl-$CD_3$ at room temperature is longer than for the other three trityls [11], and since nuclear hyperfine interactions are weaker for trityl-$CD_3$, $T_m$ at room temperature is longer than for the other trityls.

The temperature dependence of $T_1$ measured by inversion recovery at X-band between 77 and 293 K is similar for the four radicals (Fig. 3). For trityl-$CD_3$ in $D_2O$–glycerol-$d_8$, values of $T_1$ were indistinguishable from values in natural isotope abundance solvents, which indicates that solvent nuclei do not play a significant role in spin–lattice relaxation. It is noteworthy that melting of the glassy solvent, which has a dramatic effect on $T_m$, has little impact on $T_1$. The weak dependence of $T_1$ on molecular mobility indicates that molecular tumbling is not an effective $T_1$ relaxation process for the trityls at this microwave frequency. The

fit line was calculated for trityl-$CD_3$ including data between 20 and 77 K [11] that are not shown in Fig. 3. The model includes the Raman process, which dominates up to about 100 K, and a local mode that makes significant contributions at higher temperatures. Neither of these processes is predicted to be frequency dependent [10]. For each of the four trityls, the values of $T_1$ at W-band at 100 K were about 20% shorter than those at X-band (Table 1). Although the samples for X-band experiments were deoxygenated, the samples for W-band experiments were not deoxygenated. To test whether deoxygenation contributed to the difference between the X-band and W-band measurements, X-band values of $T_1$ for OX63 at 100 K in air-saturated solutions were measured. The values are indistinguishable from the values in deoxygenated solution, which demonstrates that deoxygenation does not impact $T_1$ for these glassy-solution samples. Room temperature values of $T_1$ measured for trityl-$CD_3$ by long-pump saturation recovery agreed well with values measured by inversion recovery, which indicates that spectral diffusion processes do not contribute to those recovery curves. Spectral diffusion may be a more significant contribution at W-band than at X-band because the spectrum is broader and $B_1$ is not large enough to encompass the complete spectrum at W-band. At 100 K, the use of longer, more selective pulses for the W-band inversion recovery measurements gave shorter values of $T_1$ than were observed with shorter pulses, which suggests that spectral diffusion may contribute to the recovery curves. However, the values obtained at X-band and W-band are similar enough to support the assignment of frequency-independent mechanisms of relaxation.

## Acknowledgements

The trityl radicals were a generous gift from Nycomed Innovations AB to Howard Halpern, University of Chicago, who supplied the samples for this study. We thank Richard W. Quine and George A. Rinard for their assistance with the S-band and 250 MHz spectrometers and resonators. This work was supported in part by NIBIB EB002807 and P41-EB002034.

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