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SATURATION RECOVERY EPR OF SQUARE PLANAR CUPRIC COMPLEXES

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EPR saturation recovery (SR) measurements for a square planar cupric complex, $CuKTSM_2$, and derivatives of $CuKTSM_2$ in different solvents have been made at X-band at liquid helium temperatures (10 – 30 K). Whether single or multiple exponential SR signals were obtained depended on the solvent. In the hydrocarbon hydrophobic paraffin oil solvent, single exponential SR signals were observed, while in the polar water/DMSO mixture, SR signals showed double exponential decays. It is hypothesized that the multiexponential SR signals reflect different axial ligation of the cupric complexes.

INTRODUCTION

Sometimes multiexponentials fit saturation recovery (SR) data for cupric complexes better than a single exponential. We have reported mutiexponentials from SR data for several samples (Pfenninger, Antholine, Barr, Hyde, Kroneck & Zumft, 1995, Froncisz & Antholine, 1996). One of the samples, CuBlm (a cupric complex of the drug bleomycin) was fit to a double exponential (Pfenninger *et al.*, 1995), but, as shown here, better fits can be obtained with a triple exponential (Fig. 1). For this complex the CW EPR spectrum appears to be a single species. In our previous work we avoided explaining multiexponential fits by invoking a stretched exponential, which has one less free parameter than the double exponential model (Pfenninger *et al.*, 1995). The stretched exponential is a convenient way to characterize SR signals when there is an unknown distribution of spin-lattice relaxation times (T_1). Others usually report single exponential SR decays for cupric complexes (for example see Fig. 13 in Eaton and Eaton, 2000). Sometimes they avoid multiexponential SR decays by altering the solvent, for example, by increasing the glycerol content (Brian Bennett, private communication). The purpose of this study is to investigate the origin of the multiexponential SR decays and to determine what can be gained from multiexponential data.

MATERIALS AND METHODS

$CuKTSM_2$ and CuKTS (see Fig. 2 for chemical structure) were generously supplied by Dr. David H. Petering (University of Wisconsin-Milwaukee). H_2O was deionized and DMSO and paraffin oil were reagent grade.

CW EPR spectra were recorded on a Varian E9. The saturation recovery experiments were performed on a pulse X-band EPR spectrometer with a TE_{102} cavity, which is located at the National Biomedical EPR Center, as described previously (Pfenninger *et al.*, 1995). All SR signals were recorded at the crossover point in the g_1 region. Experimental conditions: pump power, 125 mW; pump pulse width, 200 μs; trigger delay after the pump pulse, 4 μs; observing power, 1.2 mW. Temperature was maintained by pushing helium with a Helitron flow system comprised of a transfer line and a digital indicator/controller from Air Products (Allentown, PA) through a quartz dewar. For data acquisition the sampling interval was 32 ns. Typically 2048 decays were averaged with 32768, 16384, or 8192 data points per decay, depending on sample temperature. At the same conditions off-line decays were recorded and subtracted from those on-line for baseline correction. The SR signals were fit by single, double, or triple exponentials using IGOR Pro 4.02A program and compared.

RESULTS

Our first goal was to obtain SR data for a cupric complex that fit a single exponential. In previous work, we used $CuKTSM_2$ (3-ethoxy-2-oxobutyraldehde bis(N4,N4-dimethylthiosemicarbazonato)-Cu(II)) as a probe of membrane structure and dynamics at room temperature (Subczynski, Antholine, Hyde & Petering, 1987; Subczynski, Antholine, Hyde & Kusumi, 1990) and also studied its motion and orientation in frozen solution

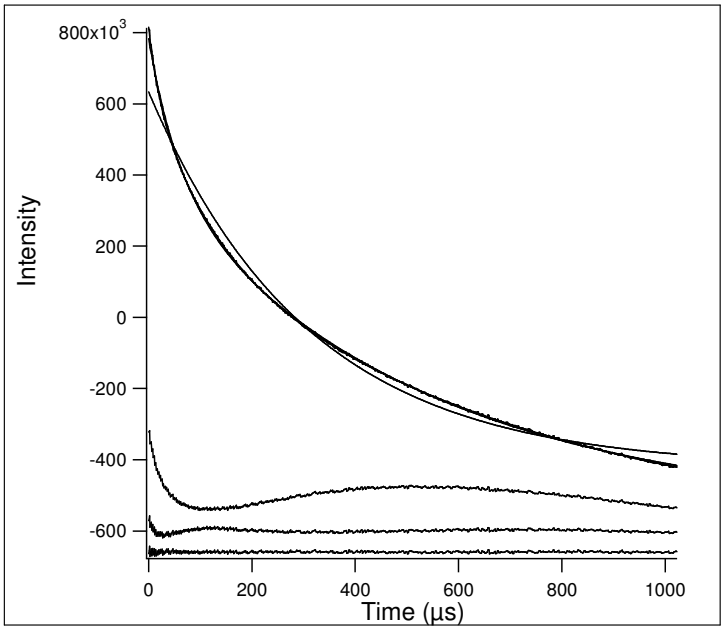

Fig. 1. SR signal of the square pyramidal, type 2 complex, cupric bleomycin recorded at 16 K. The curves superimposed on the SR signal are from single, double and triple exponentials. Residual curves are from single (top, T_1 = 310 μs), double (medium, T_1' = 520 μs and T_1" = 60 μs) and triple (bottom, T_1' = 690 μs, T_1" = 110 μs, T_1"' = 20 μs) exponential fits.

(Pasenkiewicz-Gierula, Antholine, Subczynski, Baffa, Hyde & Peterning, 1987) and at higher temperatures (up to 120°C) in paraffin oil and lipid bilayer membranes (Pasenkiewicz-Gierula, Subczynski & Antholine, 1997). We reasoned that $CuKTSM_2$ dissolved in paraffin oil (0.5 mM) was a good sample because paraffin oil as a pure hydrocarbon solvent is least likely to provide axial ligands for $CuKTSM_2$. The X-band EPR spectrum for $CuKTSM_2$ in frozen solution with well resolved nitrogen superhyperfine structure consisted of two signals, one from $^{63}CuKTSM_2$ (69%) and the other from $^{65}CuKTSM_2$ (31%) (Fig. 3). A single exponential fit the SR signal and a double exponential fit did not improve the fit (Fig. 4). T_1 is 540 μs for the single exponential fit and 540 μs (weight 78%) and 550 μs (weight 22%) for the double exponential fit at 11.7 K. Single exponential fits were obtained over the temperature range from 11 K to 30 K (see Table 1 for T_1 values). To show that trapped oxygen did not affect the T_1 values, the solution of $CuKTSM_2$ in paraffin oil was saturated with nitrogen or oxygen at room temperature and than frozen in the EPR tube. The SR signals for $CuKTSM_2$ in the presence and absence of oxygen were similar, giving practically the same T_1 values.

SR signals for CuKTS (3-ethoxy-2-oxobutyraldehyde bis(thiosemicarbazonato) Cu(II)) dissolved in DMSO and diluted in H_2O (DMSO/H_2O = 1/10 v/v) were best fit by two exponentials (Fig. 5). CuKTS was more soluble in polar solvents, but the concentration was keep low (0.3 mM) in order to prevent aggregation, which sometimes is not seen as a precipitate. The CW EPR spectrum for CuKTS consisted of two signals (resolved in the g_{II} region, but poorer resolution of the lines in the g_{I} region (Fig. 3)), which was also an indication of more than a single species.

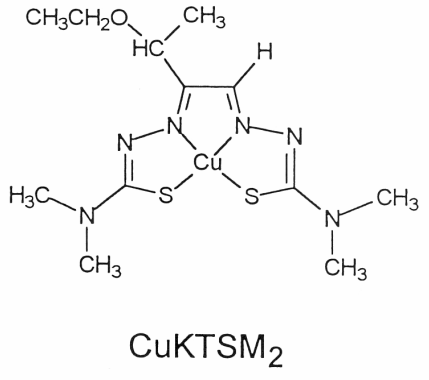

Fig. 2. Chemical structure of $CuKTSM_2$. In CuKTS .the four methyls on nitrogen atoms are replaced by four hydrogen atoms.

Table 1. T_1 values for $CuKTSM_2$ in paraffin oil at different temperatures

Temp. (K)	11.7	15.9	20.5	24.0	30.0
T_1 (μs)	530	380	270	180	130

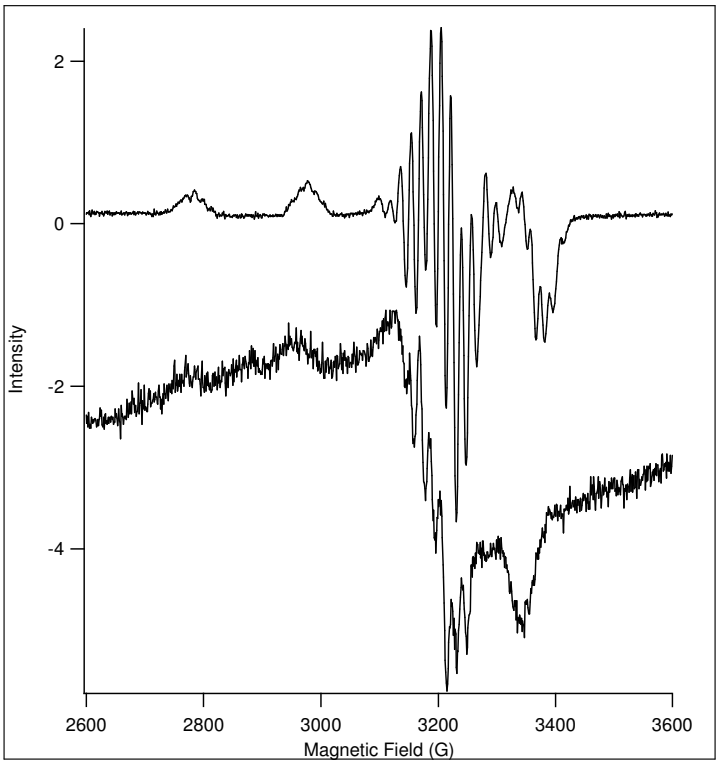

Fig. 3. CW EPR spectra of $CuKTSM_2$ in paraffin oil (top) and CuKTS in DMSO/H2O = 1/10 (bottom) recorded at 11 K.

DISCUSSION

Often EPR SR signals are best fit by multiexponentials even though the CW EPR spectrum appears to arise from a single site. For example, the SR EPR for CuBlm (Fig. 1), $Cu(catechol)_2$ (Froncisz & Antholine, 1996), CuN_2OR (Pfenninger *et al.*, 1995), and numerous other cupric complexes, for which the data are not published by us, are best fit by multiexponentials. Detection of multiexponential SR signals is a method to detect species that are not resolved by CW EPR.

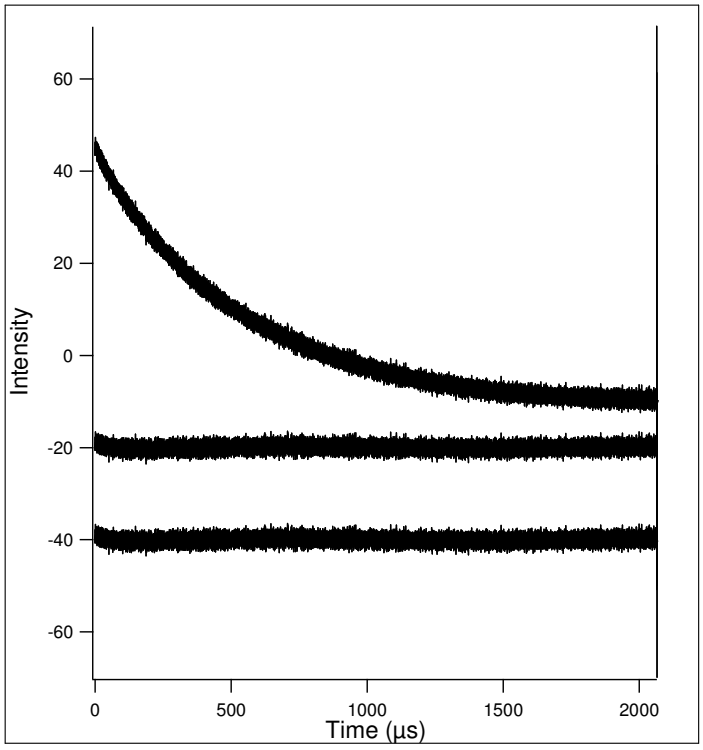

Fig. 4. SR signal of $CuKTSM_2$ in paraffin oil at 11.7 K. Fitting the trace to a single-exponential mode (time constant 540 µs, top residual line) is good. The fit to a double-exponential mode (time constants of 540 and 550 µs, bottom residual line) does not improve the fit

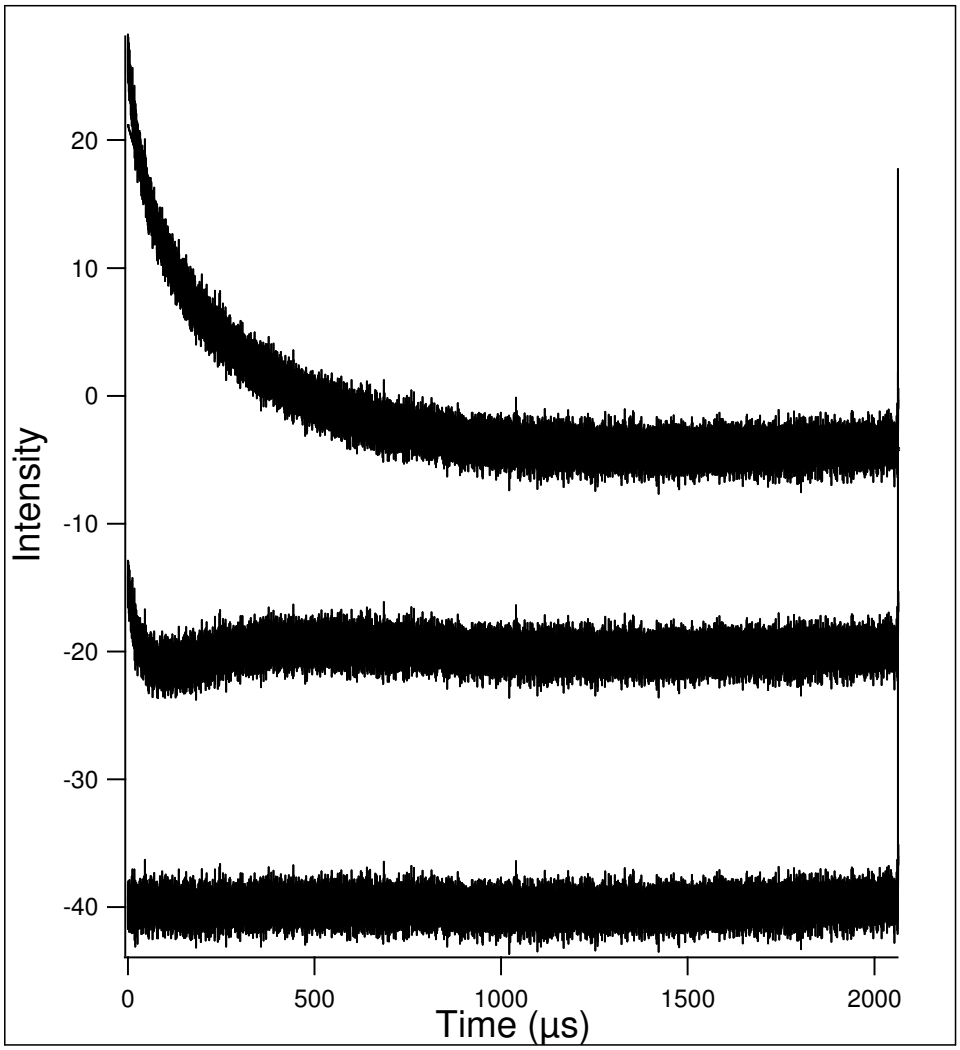

Fig. 5. SR signal of CuKTS in DMSO/H2O mixture at 11.0 K. Fitting the trace to a single exponential mode, is unsatisfactory, as shown by the residual (top). The fit using a double-exponential mode (time constants of 290 µs (weight 70%) and 30 µs (weight 30%), bottom residual line) is good.

It is more problematic to show that multiexponential SR signals are related to axial ligation. On the positive side, axial ligands, for cupric ions in samples where multiexponential SR signals are obtained, are solvent molecules. All our SR data to date are fit by one, two, or three exponentials, which correlates with zero, one, or two axial ligands. Finally, changing the solvent to that in which the axial ligation is expected to be limited (in this case dissolving $CuKTSM_2$ in paraffin oil), results in a single exponential.

Acknowledgement

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