II B. CENTER HIGHLIGHTS

Research Highlight #142

NARS Spectroscopy in Square Planar Copper Complexes Having Nitrogen Ligands
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Introduction: The method of NARS spectroscopy was recently introduced by us [1]. It was developed in the course of work to improve EPR methodology to measure interspin distances between pairs of nitroxide radical spin labels. We realized that it would be preferable to detect pure absorption spectra rather than first harmonic derivative-like spectra obtained using magnetic field modulation. This is because field modulation always broadens EPR lines and sacrifices signal intensity. In this research highlight, we report results of a study designed to broaden the range of applications of the NARS method to include EPR of transition-metal complexes. We used the square planar Cu^{++} complex that is formed by four in-plane histidine ligands (Fig. 1A). Although our thrust here is to use this complex as a model system that could extend NARS methodology, it is also a model for Type II copper-binding proteins of biomedical interest including prion protein, β-amyloid, α-synuclein, and ceruloplasmin. The spectrum is about 800 G wide and was assembled from 170 segments of 5 G. Each segment was formed from signal averaging 100,000 individual segments acquired at a 2600 Hz rate.

Methods: An acquired spectrum is shown in Fig. 1B. Possible evidence of nitrogen superhyperfine structure can barely be seen as slight inflections. However, the signal-to-noise ratio is exceptionally high. We came to realize that first harmonic derivative-like spectra are required for the EPR study of this molecule. Some years ago, we developed a post-processing algorithm called pseudomodulation [2] that could be applied to a spectrum such as that of Fig. 1B and would mimic with mathematical precision the effect of experimental application of field modulation. The input to the algorithm is the desired field modulation amplitude, and the output is the desired harmonic. In this report, we studied the application of this algorithm to the spectrum of Fig. 1B over a wide range of modulation amplitudes. In all cases, we selected the first harmonic for display.

Results: Figure 1C shows the result of application of a 5 G pseudomodulation amplitude to the spectrum of Fig. 1B. Nitrogen superhyperfine structure, which was known to be about 13 G, is clearly evident. This information was present in the data of Fig. 1B but not visually apparent. Figures 1D-G are expansions of our regions of spectrum 1C, but were obtained by applying other pseudomodulation amplitudes to Fig. 1B. To make Fig. 1F, 18 spectra were acquired in 1 G intervals from 1 to 18 G, integrals were normalized, and overlapped—noting that

Figure 1: Pseudomodulation of Cu^{++} EPR spectra from NARS spectrum B.

some of these 18 spectra were omitted for clarity. Spectral broadening and signal loss were evident even at 1 G amplitude. Nevertheless, the quality of the 1 G spectrum was sufficiently high to allow spectral analysis at high resolution. We recently published an analysis of this portion of the spectrum [3], which was obtained in the classical way. Figures 1D and G were from the $m_I = +/-3/2$ g-parallel portions of the spectrum using 20 G pseudomodulation amplitude. It is hypothesized that the shapes of these lines will be very sensitive to distributions of bonding parameters that result in strain broadening. In Fig. 1E, which is from the $m_I = -1/2$ g-parallel region, strain broadening cancels and the nitrogen superhyperfine coupling is revealed. The dashed lines assume four nitrogens, which is known from Fig. 1A to be correct. Agreement between model and data is very good.

Conclusion: Spectra C-G were obtained from a single spectrum, B, by post-processing and are internally consistent. The displays were selected from numerous calculated spectra obtained at other modulations. Acquisition of equivalent experimental spectra using conventional magnetic field modulation is impractical and perhaps impossible because of the necessity for long-term stability of the spectrometer.
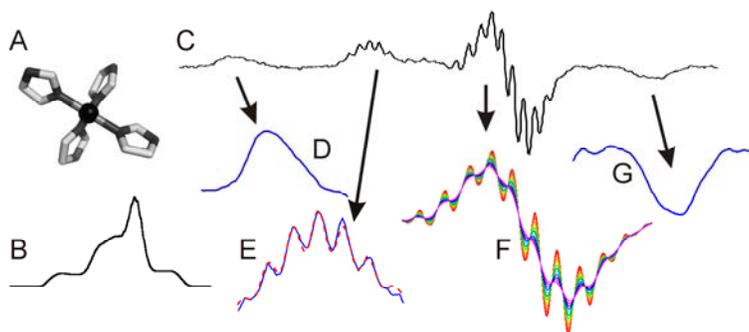
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