

Research Highlight #154

Spin-label CW microwave power saturation and rapid passage with triangular non-adiabatic rapid sweep (NARS) and adiabatic rapid passage (ARP) EPR spectroscopy

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Introduction: Acquisition of pure absorption electron paramagnetic resonance (EPR) spectra rather than the derivative-like displays that are obtained through use of magnetic field modulation and phase sensitive detection has been a long sought goal of James Hyde. That goal has been realized in the last few years, and four papers have been published,^{1,2,3,4} including another one in the last grant period.⁵ The method is called non-adiabatic rapid sweep (NARS) spectroscopy.

Methods & Results: Figure 1 shows NARS spectra from a nitroxide radical spin label (SL) that were obtained in the very slow rotational diffusion limit at two incident power levels, one eight times higher than the other, under conditions of the onset of microwave power saturation. Figure 2 was produced by multiplying the lower power spectrum (P_L) by $8^{1/2}$ since, in the absence of power saturation, the EPR signal is expected to vary as the square root of the incident power. The difference [(slightly saturated spectrum) – $8^{1/2}$ (low power spectrum)] is plotted in Fig. 2a. Figure 2b was created by applying a moving difference (MDIFF) algorithm to Fig. 2a to create a “derivative-like” display. The detailed spectral information that must exist in the spectra of Fig. 1 was extracted by a simple signal-processing method. These two figures establish that “turning points” in the spectrum (i.e., points A and C) saturate more readily than regions between turning points (e.g., point B). It is now clear that T_1 -values do not vary across the spectra – it is the variation of the T_2 -values that is responsible for the high spectral resolution of Fig. 2b.

Figure 3 was extracted from the paper in which Hyde and Dalton introduced the method of saturation-transfer EPR spectroscopy.⁶ They used the adiabatic rapid passage method under conditions of very slow rotational diffusion. The similarity of Figs. 2a and 3 is apparent, even though the methods of signal acquisition were completely different. The differences are also apparent: the remarkable resolution of Fig. 2 is lost in Fig 3.

Implications & Discussion: These new results using the NARS continuous wave saturation method inspires a new design of experiment using the pulse method of saturation recovery (SR) EPR. Both NARS and SR make measurements of the EPR signal intensity at a spectral point, whereas magnetic field modulation blurs spectral information through use of 5 G field modulation amplitude. The difference in spectral resolution comparing Figs. 2 and 3 is attributed to this fact. In addition, pulsed ELDOR, which is the measurement of saturation transfer from one spectral point to another, may provide the direct observation of very slow rotational diffusion, nitrogen nuclear relaxation, chemical exchange, physical exchange between lipid phases, and Heisenberg exchange. High-resolution spectra, as shown in Fig. 2b, provide a basis for separation of longitudinal relaxation (a T_1 process) and transverse relaxation (a T_2 process). The long-term goal is to characterize anisotropic rotational diffusion of membrane-bound proteins and single eye lenses at very low volumes.

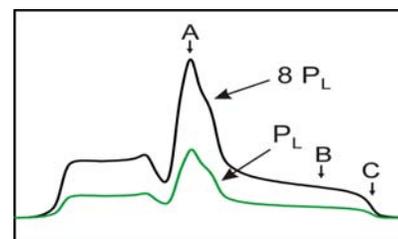


Fig. 1. NARS spectra of very slow tumbling SL at two power levels.

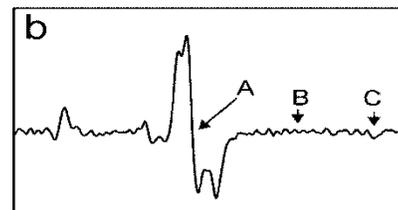
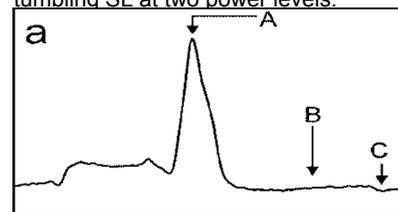


Fig. 2. A. Difference spectrum from data of Fig. 1. B. MDIFF of Fig. 2a.

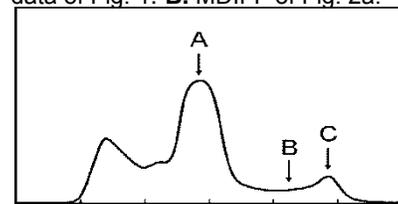


Fig. 3. First harmonic out-of-phase dispersion spectrum of a very slow tumbling SL.

¹ Hyde JS, Bennett B, Kittell AW, Kowalski JM, Sidabras JW. (2013) Moving difference (MDIFF) nonadiabatic rapid sweep (NARS) EPR of copper(II). *J Magn Reson.* 236:15-25. PMID: PMC3919454.

² Kittell AW, Camenisch TG, Ratke JJ, Sidabras JW, Hyde JS. (2011) Detection of undistorted continuous wave (CW) electron paramagnetic resonance (EPR) spectra with non-adiabatic rapid sweep (NARS) of the magnetic field. *J Magn Reson.* 211(2):228-33. PMID: PMC3148028.

³ Kittell AW, Husted EJ, Hyde JS. (2012) Inter-spin distance determination using L-band (1-2 GHz) nonadiabatic rapid sweep electron paramagnetic resonance (NARS EPR). *J Magn Reson.* 221:51-6. PMID: PMC3957363.

⁴ Kittell AW, Hyde JS. (2015) Spin-label CW microwave power saturation and rapid passage with triangular non-adiabatic rapid sweep (NARS) and adiabatic rapid passage (ARP) EPR spectroscopy. *J Magn Reson.* 255:68-76. PMID: PMC4441560.

⁵ Ibid.

⁶ Hyde JS, Dalton L. (1972) Very slowly tumbling spin labels: adiabatic rapid passage. *Chem Phys Lett.* 16:568-572.