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Short Note

Optimal EPR detection of weak nitroxide spin adduct and ascorbyl free radical signals

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Summary

We have investigated the optimal nominal power settings for the electron paramagnetic resonance detection of typical nitroxides, nitroxide spin adducts, and the ascorbyl free radical. In room temperature aqueous solution, we find that, for all the nitroxides examined, saturation effects begin at approx. 25 mW nominal power with maximum signal intensity achieved at approx. 100 mW power when using a TM_{110} cavity. For the ascorbyl free radical, we find that saturation effects begin at approx. 16 mW nominal power and that maximum peak-to-peak signal amplitude is obtained at approx. 40 mW microwave power. For the ascorbyl free radical, we find that a modulation amplitude of approx. 0.65 G yields the maximum signal height for the doublet signal. This information will help researchers maximize the EPR signal height of minimally detectable free radicals such as encountered in biological systems.

Key words: Electron paramagnetic resonance; Spin trapping; Ascorbyl radical; Free radical; Nitroxide radical

Introduction

Electron paramagnetic resonance (EPR) spin trapping is now widely used to study free radical formation in chemical, biochemical, and biological systems [1]. However, in room (or physiological) temperature biochemical and biological systems, the EPR detection of weak free radical signals is problematic. Free radical species, even if spin trapped, often have a short lifetime that precludes extensive use of signal averaging. Furthermore, it is often desirable to monitor changes in EPR signals with time. Thus, it is imperative that optimal instrument settings be used with each EPR scan to achieve the best spectrum signal-to-noise ratio. Therefore, we have investigated the nominal microwave power settings that will

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achieve the maximum peak-to-peak signal amplitude for typical nitroxides, such as nitroxide spin adducts, and for the ascorbyl free radical.

Materials and Methods

3-Carboxyproxyl (3-CP), 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), *N*-tert-butyl-α-phenyl-nitrone (PBN) and 4-amino TEMPO were obtained from Aldrich Chemical Company (Milwaukee, WI). 1-(4-Pyridyl-1-oxide)-*N*-tert-butyl-nitrone (POBN), 2-methyl-2-nitrosopropane (MNP), and ascorbic acid were obtained from Sigma Chemical Company (St. Louis, MO). DMPO was purified with charcoal and stored as a 1.0 M aqueous solution before use [2]. ESR spectra were collected with a Bruker ESP-300 electron spin resonance spectrometer using a TM_{110} cavity and an aqueous flat cell. We used nominal power settings that ranged from 0.8 to 200 mW. When necessary, all signal heights were corrected for signal degradation over time.

The DMPO/$\cdot$OH spin adduct was formed with the aid of a Fenton system: DMPO, 10 mM; H_2O_2, 0.2 mM; Fe(II), 0.1 mM. The methyl radical spin adducts of DMPO, MNP, PBN, and POBN were formed with a Fenton system that also included DMSO, 5 mM. The ascorbyl free radical was detected in a metal-free 50 mM pH 7.8 phosphate buffer that contained 10 mM ascorbic acid [3]. This solution produced an ascorbyl radical signal whose intensity varied minimally over the time of the experiment. For each species examined, the low field peak was used to determine the peak-to-peak signal amplitude as the power was varied. All signal heights were normalized to percent maximum value for each species.

Results and Discussion

When there is no saturation of the sample, the EPR signal amplitude is proportional to $(\text{power})^{1/2}$. For the ascorbyl free radical doublet spectrum ($a^H = 1.8$ G), we find that saturation effects begin at ≈ 16 mW and maximum ESR signal height occurs at ≈ 40 mW power (Fig. 1). The choice of modulation amplitude to use for detecting weak EPR signals is usually a compromise between sensitivity and faithfulness of line shape. Each line of the ascorbyl radical doublet is actually a triplet of doublets * [4]; therefore, resolution has already been sacrificed for sensitivity. We find that a modulation amplitude of approx. 0.65 G maximizes the ascorbyl free radical doublet peak-to-peak signal amplitude.

Our EPR power saturation results with the stable nitroxides 3-CP and 4-amino TEMPO in room temperature aqueous solution show that saturation effects begin to occur at approx. 25 mW and that maximum EPR signal height is achieved at approx. 100 mW nominal power (Fig. 2). The results we observed with the

* The EPR spectral parameters for the ascorbyl free radical in aqueous solution have been reported to be [4]: g-factor = 2.00518; $a^{H4} = 1.76$ G; $a^{H6}(2) = 0.19$ G; $a^{H5} = 0.07$ G.

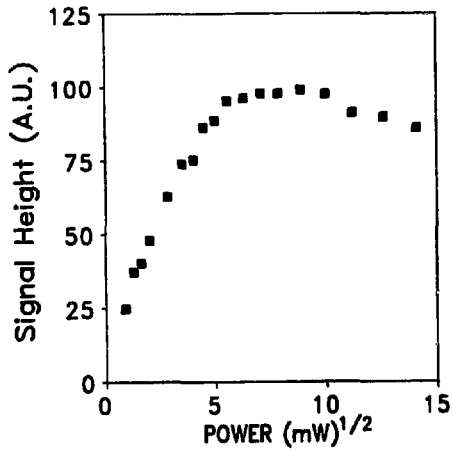

Fig. 1. Power saturation curve for the ascorbyl free radical. Signal heights are normalized to percent maximum value. Instrument settings were: modulation amplitude, 0.65 G; scan rate, 10 G/167 s; time constant, 0.167 s.

nitroxide spin adducts DMPO/·OH, DMPO/·CH3 (Fig. 3), MNP/·CH_3, PBN/·CH_3, and POBN/·CH_3 (Fig. 4) were similar to those observed for 3-CP and 4-amino TEMPO.

A survey of spin trapping papers shows that most researchers use nominal power settings of 10 or 20 mW to collect room temperature spin adduct EPR solution spectra. Our results demonstrate that researchers have available to them instrument parameters that can easily increase the EPR signal amplitude of the typical spin trapping experiment. Although some saturation effects will be present, researchers can use higher power settings. If the absolute concentration of the free

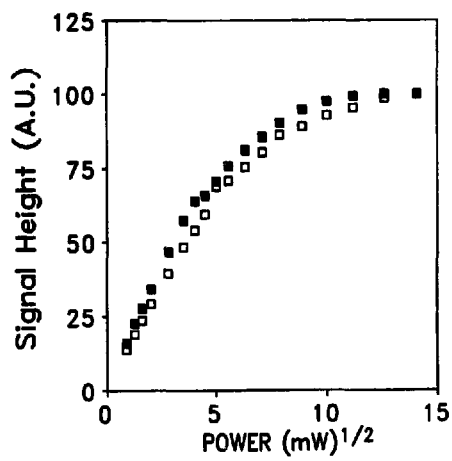

Fig. 2. Power saturation curves for 3-CP, ■, and 4-amino-TEMPO, □. The data were gathered using approx. 50 μM aqueous solution of each nitroxide. Signal heights are normalized to percent of maximum value of each curve. Instrument settings were: modulation amplitude, 0.5 G; scan rate, 50 G/167 s; time constant, 0.17 s.

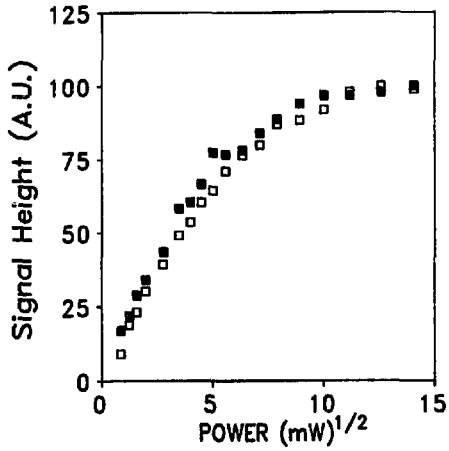

Fig. 3. Power saturation curves for DMPO/·OH: □; and DMPO/·CH_3: ■. Signal heights are normalized to percent maximum value.

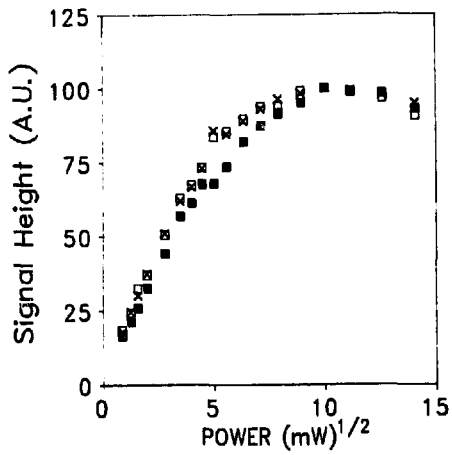

Fig. 4. Power saturation curves for PBN/·CH_3: □; POBN/·CH_3: ×; and MNP/·CH_3: ■. Signal heights are normalized to percent maximum value.

radicals being observed is desired, then the power saturation curve must be considered. These results allow researchers to do this.

Acknowledgements

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