Application of Over Magnetic Field Modulation in EPR Oximetry

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Abstract
The over-modulation technique was applied to EPR oximetry experiments. Modulation amplitudes in the range of 2-18 times the intrinsic linewidth (half-width-at-half-maximum, HWHM) of the oxygen probe were applied to increase the signal-to-noise ratio of spectra. The intrinsic linewidth of the probe at different oxygen concentrations was accurately extracted through curve fitting from the modulation-broadened spectra.

Introduction
In a standard EPR spectrometer, magnetic field modulation followed by phase-sensitive detection has been universally used. This technique increases EPR signal but also broadens the lineshape of EPR spectrum. If accurate linewidth information is desired, small modulation amplitude needs to be used to limit the lineshape distortion. The restriction of using low modulation amplitude results in low signal-to-noise ratio EPR spectra and makes direct linewidth measurements infeasible in applications where the EPR signal is weak. Recently, over-modulation technique has been studied (1, 2) and used to improve the accuracy of linewidth measurements in EPR spectral-spatial imaging experiments (3). In this work, we applied the over-modulation technique in EPR oximetry experiments.

Method
The oxygen sensing particulate lithium octa-n-butoxy-substitued naphthalocyanine (LiNc-BuO) was used in the experiments (4). Modulation amplitude up to 4 G was applied to increase the signal-to-noise ratio of the EPR spectra. The over-modulation model described in (1, 2) was used to fit the enhanced EPR spectra and therefore to extract the intrinsic linewidth information of LiNc-BuO at different oxygen concentrations. The oxygen sensitivity curve for LiNc-BuO was then obtained.

Experiments and Results
A small amount (~10 μg) of LiNc-BuO microcrystals was encapsulated in a 0.8 mm diameter gas-permeable Teflon tube (Zeus Industrial Products, Orangeburg, SC, USA). The sealed sample was inserted into a 3 mm quartz EPR tube with both ends open. Desired compositions of 0%, 0.5%, 2.5%, 5% and 20.9% (room air) of oxygen and remaining nitrogen were obtained from proxy air. A pre-calibrated gas flow meter (Cole-Parmer, Vernon Hills, IL, USA) was used, and the mixture was sent through gas-impermeable silicon tubes into the EPR tube. The EPR tube was placed into the TM_{110} microwave cavity (X-band). All the measurements were carried out after equilibrating the sample with the gas mixture for at least 10 min. The flow rate of the gas mixture was maintained at 2 L/min. The total pressure inside the EPR tube was maintained at 760 mmHg (atmospheric pressure). For each oxygen concentration, the modulation amplitude was varied from 0.01 G to 3.98 G in 32 steps. Three different power levels, 5 μW, 50 μW and 500 μW, were applied at each oxygen concentration. Therefore, a total of 480 spectra were acquired but only those spectra acquired at modulation amplitude not less than twice the intrinsic linewidth (HWHM) were chosen to extract the intrinsic linewidth through curve fitting. Other experimental parameters were as the following: scan width = 10 G, time constant = 5.1 ms, scan time = 10.5 s. Fig. 1 shows fitting result of an experimental spectrum of LiNc-BuO acquired at 5% oxygen using modulation of 2.24 G (the intrinsic peak-to-peak linewidth is 0.567 G). The fitting result is overlapped with the experimental spectrum and no systematic fitting error is seen. Fig. 2 is the results of the extracted intrinsic linewidth of LiNc-BuO at 5% pO_2. At the same oxygen concentration, the variation of fitting results is less than 10 mG, as compared to the intrinsic linewidth 0.567 G, even though large modulation amplitudes up to 4 G have been applied. Fig. 3 is the oxygen sensitivity curve of LiNc-BuO. The solid circle ● is the averaged linewidth at the same oxygen pressure but with 3 different microwave power levels. The solid line is the fitting result (R = 0.99999) that represents the sensitivity of LiNc-BuO. The slope of the sensitivity line is 7.947 mG/mmHg while the intercept is 262.87 mG. These values are consistent with the previously reported results (4) considering different batches of the sample were used in two experiments.

Conclusion
We demonstrated successfully applications of the over-modulation technique to the EPR oximetry experiments using the oxygen sensing probe LiNc-BuO. The linewith information of LiNc-BuO at different oxygen concentrations has been accurately extracted from the signal-enhanced but lineshape-distorted spectra by curve fitting using the over-modulation model. The EPR linewidth versus pO_2 curve was constructed for LiNc-BuO that was in good agreement with the previously reported values.

References
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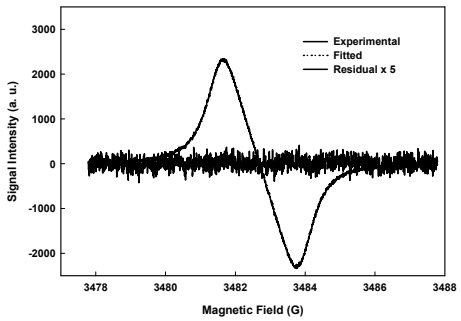

Fig. 1 Fitting experimental spectrum

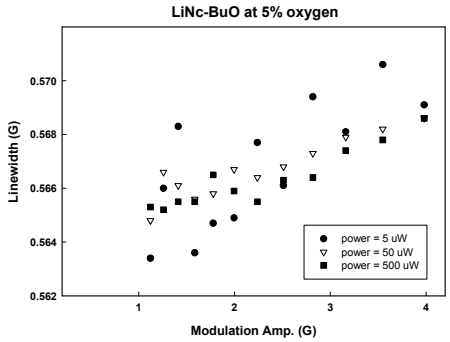

Fig. 2 Extract of intrinsic linewidth

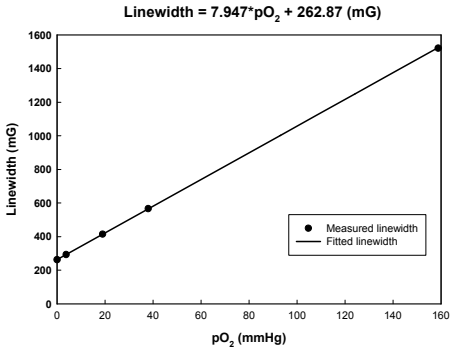

Fig. 3 Oxygen sensitivity curve of LiNc-BuO