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Application of magnetic field over-modulation for improved EPR linewidth measurements using probes with Lorentzian lineshape

Yuanmu Deng, Ramasamy P. Pandian, Rizwan Ahmad, Periannan Kuppusamy *, Jay L. Zweier

Center for Biomedical EPR Spectroscopy and Imaging, Davis Heart and Lung Research Institute, Division of Cardiovascular Medicine, Department of Internal Medicine, The Ohio State University College of Medicine, Columbus, OH 43210, USA

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Abstract

Magnetic field modulation in CW electron paramagnetic resonance (EPR) is used for signal detection. However, it can also distort signal lineshape. In experiments where the linewidth information is of particular importance, small modulation amplitude is usually used to limit the lineshape distortion. The use of small modulation amplitude, however, results in low signal-to-noise ratio and therefore affects the precision of linewidth measurements. Recently, a new spectral simulation model has been developed enabling accurate fitting of modulation-broadened EPR spectra in liquids. Since the use of large modulation amplitude (over-modulation) can significantly enhance the EPR signal, the precision of linewidth measurements is therefore greatly improved. We investigated the over-modulation technique in EPR oximetry experiments using the oxygen-sensing probe lithium octa-*n*-butoxy-substitued naphthalocyanine (LiNc-BuO). Modulation amplitudes 2–18 times the intrinsic linewidth of the probe were applied to increase the spectral signal-to-noise ratio. The intrinsic linewidth of the probe at different oxygen concentrations was accurately extracted through curve fitting from the enhanced spectra. Thus, we demonstrated that the over-modulation model is also applicable to particulate oxygen-sensing probes such as LiNc-BuO and that the lineshape broadening induced by oxygen is separable from that induced by over-modulation. Therefore, the over-modulation technique can be used to enhance sensitivity and improve linewidth measurements for EPR oximetry with particulate oxygen-sensing probes with Lorentzian lineshape. It should be particularly useful for in vivo oxygen measurements, in which direct linewidth measurements may not be feasible due to inadequate signal-to-noise ratio.

Keywords: EPR; Magnetic field modulation; Over-modulation; EPR oximetry; Simulation; Free radical

1. Introduction

Oxygen measurement has been found to be extremely important in the diagnosis and therapy of a variety of diseases such as cancer, peripheral vascular disease, wound healing and stroke [1–6]. For example, tumor oxygenation is a critical parameter in planning tumor treatments and assessing their outcomes [7]. As a non-invasive approach, electron paramagnetic resonance (EPR) oximetry offers accurate and continuous oxygen measurements over a long period of time from the same site [2,3,7,8]. In EPR oximetry [2,3,5,8–10], an oxygen sensitive paramagnetic EPR probe is placed in the site of interest. Due to the interactions between the probe and molecular oxygen, the linewidth of the EPR spectrum is increased. The oxygen concentration is calculated from the linewidth-broadening information using a standard sensitivity curve of that probe.

In standard commercial EPR spectrometers, magnetic field modulation followed by phase-sensitive detection has been universally used [11]. This technique amplifies the EPR signal and increases the signal-to-noise ratio but also can broaden the lineshape of the EPR spectrum.

* Corresponding author. Fax: +1 614 292 8454.
E-mail address: kuppusamy-1@osu.edu (P. Kuppusamy).

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Therefore, if accurate linewidth information is desired, small modulation amplitude (<30% of the intrinsic linewidth) needs to be used to limit the lineshape distortion. The restriction of using low modulation amplitude consequently limits the improvement of signal-to-noise ratio of the spectra and makes direct linewidth measurements difficult when the EPR signal is too weak.

In order to improve the linewidth measurement, a Voigt function, which is mathematically defined as the convolution of a Gaussian function with a Lorentzian function, has been used to fit the EPR spectra. Halpern et al. [12] used a sum of a Gaussian function and a Lorentzian function to approximate a Voigt EPR spectrum. They found that the Lorentzian component of the Voigt lineshape corresponded to the oxygen-induced homogenous broadening, while the Gaussian component accounted for all other inhomogeneous broadening. This model worked accurately when the modulation amplitude was less than 0.1 G (intrinsic peak–peak linewidth of the probe varied from 0.28 to 0.5 G). Peric and Halpern [13] therefore extended this study to allow large modulation amplitude, up to the intrinsic peak-to-peak linewidth of the probe. Bales et al. [14] developed Peric and Halpern's work by deriving a simple analytic expression to calculate the linewidth of the Gaussian component in a Voigt spectrum broadened by magnetic field modulation. Similar to Peric and Halpern's method, the approach by Bales et al. also required the modulation amplitude to be less than the intrinsic linewidth of the probe. Instead of using the sum function, Smirnov and Belford [15] proposed a direct convolution method to separate the Lorentzian and Gaussian components from the Voigt lineshape. They successfully observed the Lorentzian broadening of the central nitrogen hyperfine component of aqueous solution EPR spectra changing linearly with the molecular oxygen concentration. As in the previous models, a very small modulation amplitude (<30% of the intrinsic linewidth) was used to avoid the inhomogeneous linewidth broadening in their experiments.

As these studies attempted to improve the linewidth measurement by fitting the spectra based on the Voigt model, all the above approaches required the use of a small amount of modulation amplitude (up to the intrinsic linewidth of the probe) to limit the inhomogeneous line-broadening to obtain accurate linewidth information. However, as is known, increase of modulation amplitude is desirable to enhance the EPR signal. So the question is, instead of using small modulation amplitude to minimize the inhomogeneous lineshape distortion, whether we can use large modulation amplitude (over-modulation) to increase the signal-to-noise ratio of the spectra for better EPR linewidth measurements.

The effects of magnetic field modulation on the resonance lineshape have been studied back in early 1960's. Wahlquist [11] examined the effects of modulation broadening on a Lorentzian resonance line and derived a closed analytic expression. Wahlquist pointed out that when the modulation amplitude equals 4 times the intrinsic linewidth (HWHM), the EPR signal reaches the maximum. However, the importance of Wahlquist's work has not been well recognized and for decades small amount of modulation amplitude has been preferred in EPR spectroscopy experiments. Hyde et al. [16] proposed a filtering algorithm (pseudomodulation) to simulate the effect of sinusoidal magnetic field modulation. The pseudomodulation technique was successfully applied for spectral resolution-enhancement [17] and was recently justified by Nielsen and co-workers by solving the Bloch equation [18]. Robinson et al. [19,20] published an universal model to simulate the experimental EPR spectra in liquids by incorporating both the modulation amplitude and frequency. They have demonstrated that with precisely known modulation amplitude, the intrinsic linewidth could be accurately extracted through curve fitting of the over-modulated (up to 20 times its intrinsic linewidth) spectra. Based on Robinson's model, Mailer et al. [21] recently reported a remarkable precision improvement in the linewidth measurement of the deoxygenated OX031 spin probe using EPR spectral-spatial imaging.

In this work, we evaluated the over-modulation technique for application to EPR oximetry experiments. A recently developed particulate lithium octa-*n*-butoxy-substitued naphthalocyanine (LiNc-BuO) [9,22] was used for linewidth measurements. Modulation amplitude up to 18 times the intrinsic linewidth (HWHM) was applied to increase the signal-to-noise ratio of spectra. The Robinson model was then used to fit the enhanced spectra and the intrinsic linewidth information of LiNc-BuO at different oxygen concentrations was accurately extracted.

2. Theory

2.1. Modeling of over-modulation broadening

Robinson et al. [19,20] proposed a universal model to simulate the modulation broadening effect on a Lorentzian lineshape by incorporating modulation amplitude, frequency and microwave (H_1) phase simultaneously. In the following, we briefly summarize Robinson's model. The details of this model can be found in the references [19–21].

A complex Lorentzian line is defined as

$$\text{Signal}_0 = \frac{-1}{\pi a_0} = \frac{-1}{\pi(H - H_0) + i\pi R_{2e}} \tag{1}$$

where $a_0 = (H - H_0) + iR_{2e}$, H and H_0 are the magnetic field and the resonance magnetic field, respectively, and R_{2e} is the half-width at half-maximum (HWHM) of the Lorentzian absorption signal, all in Gauss. The real part of Signal_0 in Eq. (1) represents the EPR dispersion signal and the imaginary part of Signal_0 is the EPR absorption signal. When a field modulation $H_{\text{mod}} = \frac{1}{2}H_m \sin(2\pi f_m t)$ is applied, the modulation amplitude H_m, in Gauss, and the frequency f_m, in Hz, are involved in the lineshape broadening as,

$$a_1^{\pm} = a_0 \pm \frac{f_m}{\gamma} \tag{2}$$

$$g_1^{\pm} = \frac{1}{2} a_1^{\pm} \left[1 + \sqrt{1 - \left(\frac{H_m}{2a_1^{\pm}} \right)^2} \right] \tag{3}$$

$$g_0 = a_0 - \left(\frac{H_m}{4} \right)^2 \left[\frac{1}{g_1^+} + \frac{1}{g_1^-} \right] \tag{4}$$

where γ is the electron gyromagnetic ratio, $\gamma = 2.8 \times 10^6$ Hz/G. The in-phase, first-harmonic EPR signal is then expressed as

$$\text{Signal}_1 = A \cdot H_m \cdot \left[\frac{1}{g_0 g_1^+} + \frac{1}{g_0 g_1^-} \right] \tag{5}$$

where the coefficient A accounts for all the constant terms that contribute to the signal intensity. Taking the microwave phase information into account, the EPR signal has the final form,

$$\text{EPR Signal} = \text{imag}(\text{Signal}_1)\cos(\theta) + \text{real}(\text{Signal}_1) \times \sin(\theta) \tag{6}$$

By convolving the above model with appropriate hyperfine splitting, any EPR spectra of spin labels in liquids can be simulated. In this work, since the probe, LiNc-BuO, has a single-peak spectrum [9], the above mode, Eq. (6), is directly used to simulate or fit the spectra.

2.2. Fitting the over-modulated spectra

The spectrum fitting using the above model is straightforward. When the modulation amplitude H_m and frequency f_m are known, the intrinsic linewidth R_{2e}, as well as other parameters such as the coefficient A, spectrum position H_0, and the microwave phase θ can be solved by fitting the experimentally measured EPR spectrum using Eq. (6). However, it should be pointed out that, in this model, accurate extraction of linewidth information relies on the precise value of the modulation amplitude used at the sample, and this may not be available in some applications. For example, on some Bruker signal channel (www.bruker.de) based EPR spectrometers, the modulation amplitude can only be calibrated in a finite discrete steps. As a result, the modulation amplitude set by the instrument may be different from its true value measured at the sample. As will be seen in the next section, the error in modulation amplitude calibration will induce a systematical error in linewidth measurements. To overcome this problem, in our experiments, instead of using an inaccurate value, the modulation amplitude in Eq. (6) is kept as a parameter during curve fitting. To avoid the possible increase in optimization time and decrease in performance due to the introduction of an extra variable, a good start value for modulation amplitude is provided by calibrating the modulation amplitude carefully (see next section). The fitting process is then carried out iteratively by 2–4 times such that the fitting results from the previous iteration are used as the start values for the next iteration. In this way, the intrinsic linewidth is accurately extracted from the over-modulated spectra.

2.3. Modulation amplitude calibration

The modulation amplitude is calibrated according to the following equation [11],

$$\Delta H_{\text{pp(obs)}} = \Delta H_{\text{pp}} \left\{ \left(\frac{H_m}{\Delta H_{\text{pp}}} \right)^2 + 5 - 2 \left[4 + \left(\frac{H_m}{\Delta H_{\text{pp}}} \right)^2 \right]^{1/2} \right\}^{1/2} \tag{7}$$

Here ΔH_{pp} and $\Delta H_{\text{pp(obs)}}$ represent the intrinsic peak-to-peak linewidth (without modulation broadening) and the measured peak-to-peak linewidth of the broadened spectrum, respectively. During the calibration process, the modulation amplitude H_m is set. The modulation calibration coefficient (a parameter indicating the efficiency of a modulation coil in generating the modulation magnetic field) is set and a spectrum is acquired. The peak-to-peak linewidth of the acquired spectrum is then measured and compared with the value calculated according to Eq. (7). The modulation calibration coefficient is adjusted until the measured peak-to-peak linewidth from the acquired spectrum matches the calculated peak-to-peak linewidth using the above Eq. (7).

3. Results

3.1. Simulation results

The Robinson model was implemented in Matlab to illustrate the modulation effects on EPR spectra. A Gaussian lineshape (derivative) spectrum (s_G) was added to represent other non-modulation inhomogeneous lineshape broadening [12–15]. The peak-to-peak signal amplitude of the Gaussian spectrum was 0.01 (1% of the peak-to-peak signal amplitude of the Lorentzian spectrum) and its peak-to-peak linewidth was 0.05 G. A white noise (n_w) with zero-mean and variance 0.02 was also generated to represent the system noise. Fig. 1 shows examples of the over-modulated spectra and their peak-to-peak signal amplitudes. The intrinsic linewidth (peak-to-peak) in the simulation was 0.566 G, which is equal to the intrinsic linewidth of LiNc-BuO measured at 5% oxygen concentration. Fig. 1B shows that the simulated spectrum reaches its maximal value at the modulation amplitude around 2 G, i.e., 4 times of the intrinsic linewidth (HWHM). Based on Eq. (6), the intrinsic linewidth was extracted from the over-modulated spectra, shown in Fig. 2. In Fig. 2, curve A is the true linewidth and curves B–E are the extraction results of the intrinsic linewidth from different curve fitting trials. For comparison, the extracted intrinsic linewidth from the experimental spectra (LiNc-Buo at 5% oxygen) was also plotted as curve F. Fig. 2 shows that in the presence of

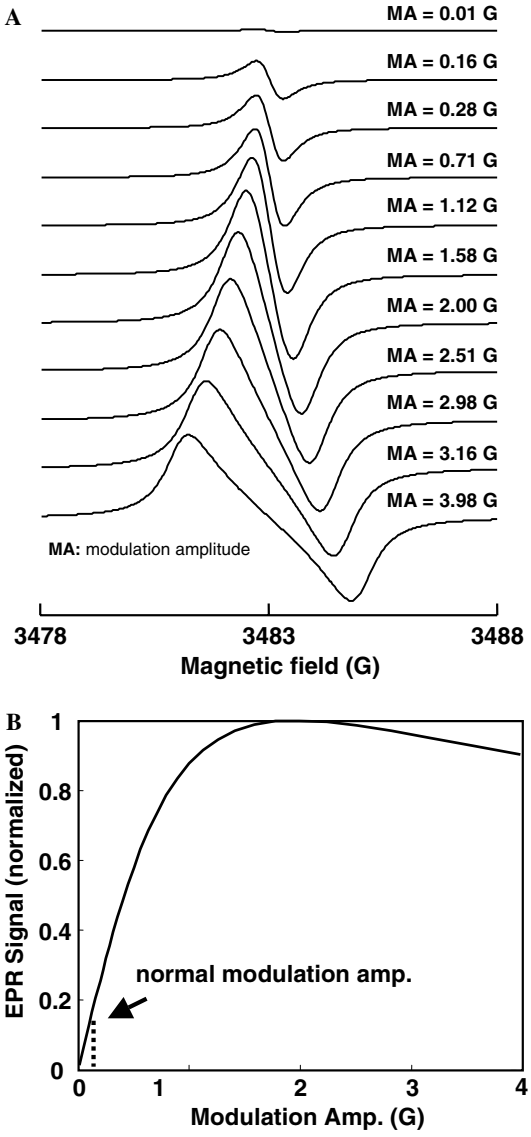

Fig. 1. Simulation of modulation effects on a Lorentzian lineshape spectrum. The intrinsic peak-to-peak linewidth used for simulation was 0.566 G, which was chosen as same as that of LiNc-BuO measured at 5% oxygen concentration. (A) Sample spectra calculated using different modulation amplitudes. (B) Peak-to-peak signal intensity changing with modulation amplitude. The signal intensity reaches its maximum when modulation amplitude is $2 \times \sqrt{3} \times 0.566 = 1.96\,\mathrm{G}$. The dotted line represents the signal amplitude when a modulation amplitude of 30% of the intrinsic linewidth is used, as required in regular EPR measurements.

other non-modulation inhomogeneous broadening, the precision of fitting results is modulation amplitude dependent. On one hand, small modulation amplitude, for example, less than 1 G in this example, caused larger fitting errors in the linewidth measurements than large modulation amplitude. On the other hand, use of accurate modulation amplitude information accelerated the convergence of the fitting results to the true linewidth (curve D) while use of inaccurate modulation caused an unacceptable systematic error in the linewidth measurements (curve E). In this example, a 2% of modulation calibration error was transferred to a systematic error of 15–20 mG, which is

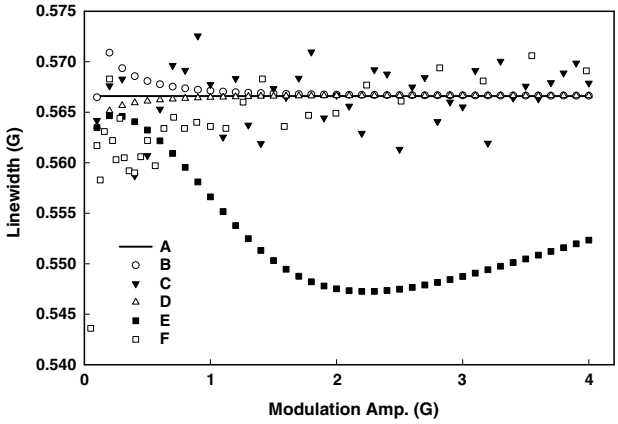

Fig. 2. Extraction of intrinsic linewidth. The spectra were simulated using Eq. (6) with the intrinsic peak-to-peak linewidth 0.566 G. A Gaussian broadening spectrum s_G and a white noise n_W were added in the simulation. The Gaussian spectrum n_G was used throughout the simulation but the white noise n_W was only used for extraction of the intrinsic linewidth with unknown modulation amplitude. See Section 3.1 for more descriptions about the noise generation. (A) Intrinsic linewidth; (B) unknown modulation amplitude + s_G; (C) unknown modulation amplitude + s_G + n_W; (D) accurate modulation amplitude + s_G; (E) inaccurate modulation amplitude (2% error) + s_G; (F) experimental spectrum of LiNc-BuO at 5% oxygen concentration (with unknown modulation amplitude).

2–3% of the intrinsic linewidth. These simulation results strongly suggested that the modulation amplitude should be large enough in order to obtain accurate linewidth information in the presence of other non-modulation inhomogeneous broadening. Thus, in our EPR oximetry experiments, we used modulation amplitude approximately twice the intrinsic linewidth (HWHM) of the probe or larger.

3.2. Experimental results

The above model was applied to the EPR oximetry experiments. A newly synthesized particulate lithium octa-*n*-butoxy-substitued naphthalocyanine (LiNc-BuO) [9] was used as the oxygen-sensitive probe. The EPR oximetry experiments were performed as follows. A small amount (~10 μg) of the LiNc-BuO microcrystals was encapsulated in a 0.8 mm diameter gas-permeable Teflon tube (Zeus Industrial Products, Orangeburg, SC) and the tube was sealed at both ends as described previously [23]. 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 Praxair (Los Angeles, CA). A pre-calibrated gas flow meter (Cole-Parmer, Vernon Hills, IL) 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) in such a way that the sample was at the center of the active volume of the resonator. 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), since the other end of the EPR tube was open to the atmosphere. For each oxygen concentration, the modulation amplitude was varied from 0.01 to 3.98 G in 32 steps. Three different power levels, 5, 50, and 500 μW, were applied at each oxygen concentration to simulate applications with different signal-to-noise ratio. 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 and data points for each spectrum = 4096.

Fig. 3 shows the relationship between the modulation amplitudes set in the instrument and their calculated values from the sample site through fitting the spectra. It is clearly seen that the calculated modulation amplitudes from the sample site (the fitting results) were slightly different from the values set by the instrument. This difference could be due to imprecise modulation calibration and/or modulation inhomogeneity in the resonator. Fig. 4 shows the fitting result of an experimental spectrum. The experimental and fitted spectra were consistent and no systematic error could be seen from the 5-fold amplified residue curve. Fig. 5A–E are the fitting results of the intrinsic linewidth of the LiNc-BuO at different oxygen concentrations and microwave power levels. At the same oxygen concentration, the variation of the fitting results is very small (<10 mG, in all cases) as compared to the intrinsic linewidth, even though large modulation amplitudes up to 4 G have been applied. The over-modulation model was robust and not sensitive to noise. For example, although a 10-fold difference in signal-to-noise ratio occurred to the experimental spectra (corresponding to 100 times difference in microwave power), accurate linewidth information has been still achieved through curve fitting. These linewidths obtained at different concentrations are consistent with the previously reported values [9,22,24].

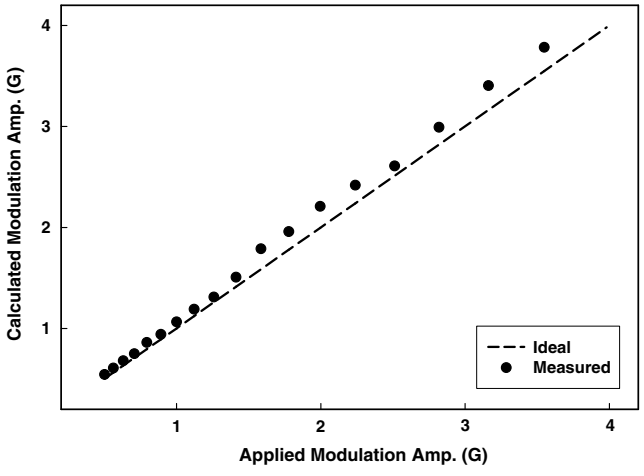

Fig. 3. Fitting results of modulation amplitudes. The dashed line represents an ideal modulation system in which the measured modulation amplitude on the sample site is exactly the same as the value set by the spectrum acquisition program. The solid circle ● is the fitting result that reflects the measured value on the sample site. Due to the imprecise modulation calibration, modulation inhomogeneity and the sample position, the difference between the preset modulation amplitude and the measured one is seen increasing as the modulation amplitude increases.

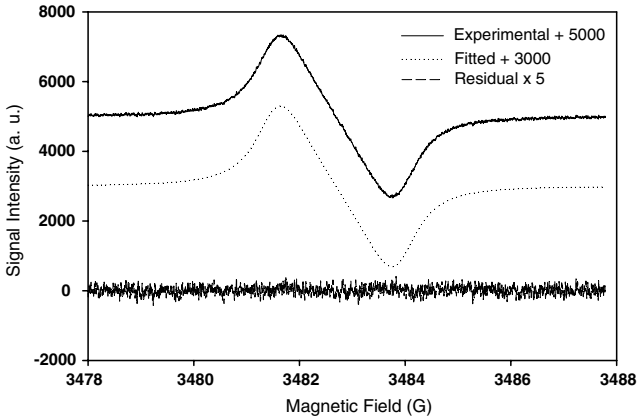

Fig. 4. Fitting result of an experimental spectrum. The solid line is the spectrum of LiNc-BuO acquired at 5% oxygen using a modulation of 2.24 G (the intrinsic peak-to-peak linewidth is 0.566 G). The dotted line is the fitting result, which is consistent with the experimental spectrum. The dashed line is the residual signal amplified by 5. No systematic fitting error is seen.

Table 1 summarizes the linewidth information of LiNc-BuO at different oxygen concentrations and different microwave power levels. No power saturation was observed at these power levels [9].

4. Discussion

Over-modulation is able to increase the signal-to-noise ratio of spectra by up to an order of magnitude (see Fig. 1B). Therefore, the accuracy of linewidth measurement can be significantly improved. There have been two over-modulation models reported in the literatures, i.e., the Wahlquist model [11] and the Robinson model [19,20]. The former used a closed analytic expression to describe the modulation effects on a Lorentzian lineshape spectrum while the latter incorporated the effects of the modulation amplitude and frequency on the complex Lorentzian lineshape. The mathematical formulae of these two models are very different. However, if we neglect the effects of modulation frequency and microwave phase in the Robinson model, both models, Eq. (6) in this paper and Eq. (6) in [11] generate exactly the same numeric spectra except a factor of $2 \cdot R_{2e}$ needs to be multiplied to the spectrum obtained by the Wahlquist model. Therefore, the conclusion drawn from the Wahlquist model that the EPR signal reaches its maximum when the modulation amplitude equals 4 times the intrinsic linewidth (HWHM) holds true for the Robinson model if only modulation

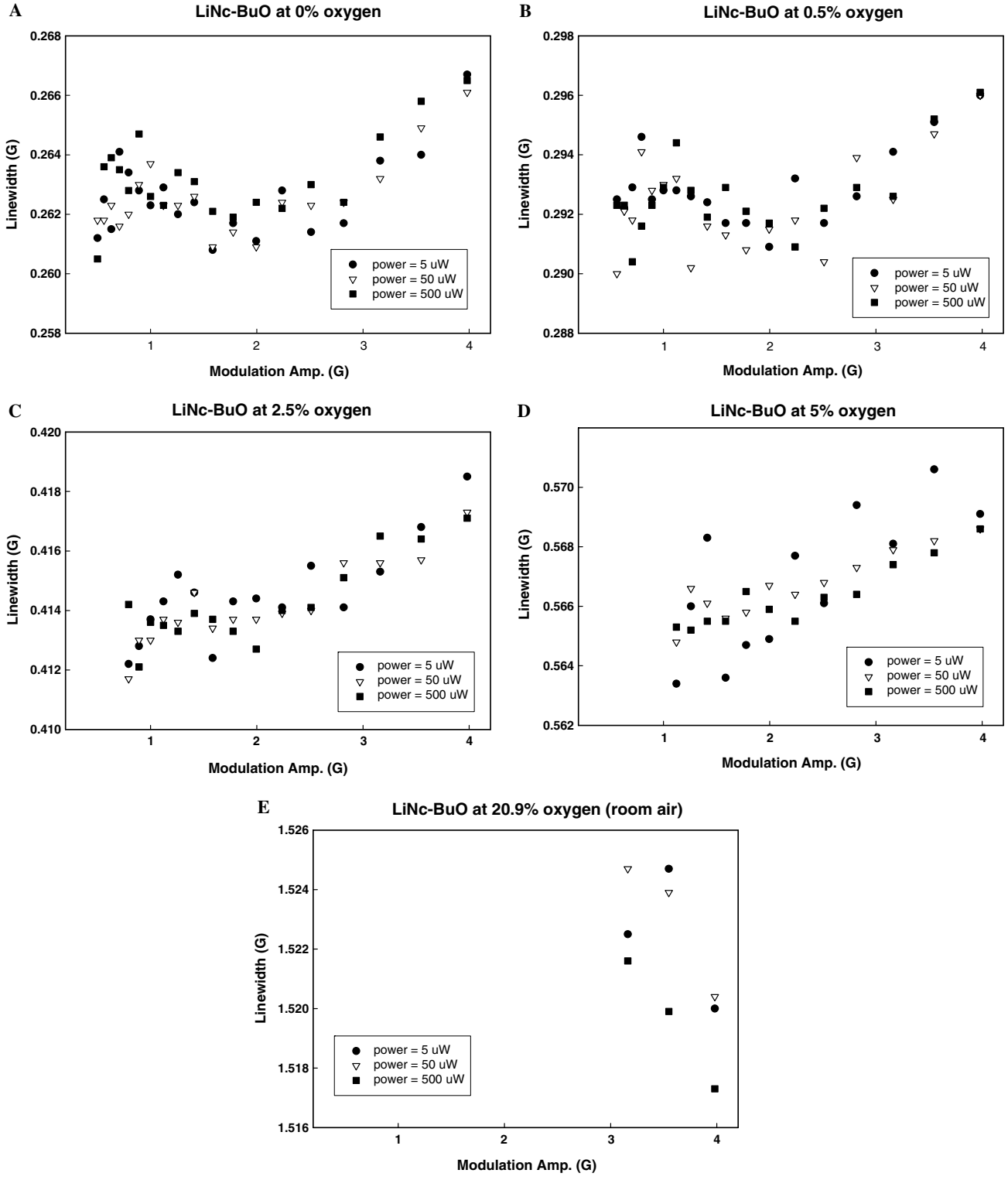

Fig. 5. Experimental results of EPR oximetry using over-modulation. (A–E) Extracted intrinsic linewidth of LiNc-BuO at different pO_2 and microwave power. Only those spectra acquired with modulation amplitude approximately 2 times the intrinsic linewidth or larger were used to extract the intrinsic linewidth information. The linewidths of LiNc-BuO at oxygen concentration 0%, 0.5%, 2.5%, 5% and 20.9% (room air) were calculated as 0.263, 0.293, 0.414, 0.565 and 1.525, in Gauss, respectively.

amplitude is involved. When modulation amplitude, modulation frequency and microwave phase are all considered, the upper limit of modulation amplitude for over-modulation EPR oximetry should be around 4 times the intrinsic linewidth (HWHM) of the probe with Lorentzian lineshape to achieve the best linewidth measurements (no closed solution is available if both modulation frequency and microwave phase are considered in fitting).

Table 1
Extraction results of the intrinsic linewidth of LiNc-BuO at different oxygen concentrations and microwave power levels

Oxygen concentration (%)	Microwave power (μw)	Mean (G)	Std	No. of spectrum used in fitting
0	5	0.2626	0.00140	19
	50	0.2625	0.00128	19
	500	0.2632	0.00142	19
0.5	5	0.2929	0.00129	18
	50	0.2923	0.00163	18
	500	0.2926	0.00140	18
2.5%	5	0.4145	0.00109	15
	50	0.4142	0.00163	15
	500	0.4142	0.00139	15
5	5	0.5668	0.00238	12
	50	0.5667	0.00112	12
	500	0.5663	0.00109	12
20.9	5	1.5224	0.00235	3
	50	1.5230	0.00229	3
	500	1.5196	0.00217	3

The fitting results from Robinson et al. [20] showed that an approximate 10% measurement error occurred when a small amount of modulation was applied. The same phenomenon was observed in our experiments. This could be explained by two reasons. First, besides the modulation inhomogeneous broadening there is other non-modulation inhomogeneous broadening co-existing in the experimental spectra due to the microwave power saturation, unresolved hyperfine structures and so on [12–15]. When the modulation amplitude was small, the effects of the modulation and non-modulation broadenings on the spectra were comparable, therefore, not separable. In this case, the fitting program could not distinguish the two inhomogeneous broadenings from each other, and consequently generated large fitting errors. When the modulation amplitude increased, the modulation broadening gradually surpassed the non-modulation broadening and the over-modulation model started working better. Accordingly, the fitting error decreased and the fitting results converged to the intrinsic linewidth. The second reason is due to the signal-to-noise ratio of the spectra. The larger the modulation amplitude, the higher the signal-to-noise ratio of the spectra and the better the fitting results. These explanations were well supported by the simulation results, as shown in Fig. 2. Therefore, in our EPR oximetry experiments, the lower limit of modulation amplitude was chosen as approximately 2 times the intrinsic linewidth of the probe (HWHM) or larger, depending on the amount of other non-modulation broadening.

5. Conclusion

We have applied the over-modulation technique to EPR oximetry experiments using 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 Robinson model. Therefore, we demonstrated that the lineshape broadening induced by oxygen is separable from the lineshape broadening induced by over-modulation and that the over-modulation technique can be used in EPR oximetry with particulate oxygen-sensing probes with Lorentzian lineshape for improved linewidth measurements. The over-modulation technique should be particularly useful for in vivo oxygen measurements, in which direct linewidth measurements may not be feasible due to the low signal-to-noise ratio of the spectra.

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References

[1] H.M. Swartz, N. Khan, J. Buckey, R. Comi, L. Gould, O. Grinberg, A. Hartford, H. Hopf, H. Hou, E. Hug, A. Iwasaki, P. Lesniewski, I. Salikhov, T. Walczak, Clinical applications of EPR: overview and perspectives, NMR Biomed. 17 (2004) 335–351.
[2] H.M. Swartz, J.F. Dunn, Measurements of oxygen in tissues: overview and perspectives on methods, Adv. Exp. Med. Biol. 530 (2003) 1–12.
[3] H.M. Swartz, T. Walczak, Developing in vivo EPR oximetry for clinical use, Adv. Exp. Med. Biol. 454 (1998) 243–252.
[4] P. Kuppusamy, R.A. Shankar, J.L. Zweier, In vivo measurement of arterial and venous oxygenation in the rat using 3D spectral-spatial electron paramagnetic resonance imaging, Phys. Med. Biol. 43 (1998) 1837–1844.
[5] H.J. Halpern, C. Yu, M. Peric, E. Barth, D.J. Grdina, B.A. Teicher, Oxymetry deep in tissues with low-frequency electron paramagnetic resonance, Proc. Natl. Acad. Sci. USA 91 (1994) 13047–13051.
[6] K.J. Liu, G. Bacic, P.J. Hoopes, J. Jiang, H. Du, L.C. Ou, J.F. Dunn, H.M. Swartz, Assessment of cerebral pO_2 by EPR oximetry in rodents: effects of anesthesia, ischemia, and breathing gas, Brain Res. 685 (1995) 91–98.
[7] B. Gallez, C. Baudelet, B.F. Jordan, Assessment of tumor oxygenation by electron paramagnetic resonance: principles and applications, NMR Biomed. 17 (2004) 240–262.
[8] J.F. Dunn, H.M. Swartz, In vivo electron paramagnetic resonance oximetry with particulate materials, Methods 30 (2003) 159–166.
[9] R.P. Pandian, N.L. Parinandi, G. Ilangovan, J.L. Zweier, P. Kuppusamy, Novel particulate spin probe for targeted determination of oxygen in cells and tissues, Free Radic. Biol. Med. 35 (2003) 1138–1148.
[10] M. Peric, B.L. Bales, Lineshapes of spin exchange broadened EPR spectra, J. Magn. Reson. 169 (2004) 27–29.
[11] C.P. Poole, Electron Spin Resonance: A Comprehensive Treatise on Experimental Techniques, John Wiley & Sons, New York, 1982.
[12] H.J. Halpern, M. Peric, C. Yu, B.L. Bales, Rapid quantitation of parameters from inhomogeneously broadened EPR spectra, J. Magn. Reson. Series A 103 (1993) 13–22.
[13] M. Peric, H.J. Halpern, Fitting of the derivative Voigt ESR line under conditions of modulation broadening, J. Magn. Reson. Series A 109 (1994) 198–202.
[14] B.L. Bales, M. Peric, M.T. Lamy-Freund, Contributions to the Gaussian line broadening of the proxyl spin probe EPR spectrum due to magnetic-field modulation and unresolved proton hyperfine structure, J. Magn. Reson. 132 (1998) 279–286.

[15] A.I. Smirnov, R.L. Belford, Rapid quantitation from inhomogeneously broadened EPR spectra by a fast convolution algorithm, J. Magn. Reson. Series A 113 (1995) 65–73.
[16] J.S. Hyde, M. Pasenkiewicz-Gierula, A. Jesmanowicz, W.H. Anderson, Pseudo field modulation in EPR spectrosocpy, Appl. Magn. Reson. 1 (1990) 483–496.
[17] J.S. Hyde, A. Jesmanowicz, J.J. Ratke, W.E. Antholine, Pseudomodulation: a computer-based strategy for resolution enhancement, J. Magn. Reson. 96 (1992) 1–13.
[18] R.D. Nielsen, E.J. Hustedt, A.H. Beth, B.H. Robinson, Formulation of Zeeman modulation as a signal filter, J. Magn. Reson. 170 (2004) 345–371.
[19] B.H. Robinson, C. Mailer, A.W. Reese, Linewidth analysis of spin labels in liquids. II. Experimental, J. Magn. Reson. 138 (1999) 210–219.
[20] B.H. Robinson, C. Mailer, A.W. Reese, Linewidth analysis of spin labels in liquids. I. Theory and data analysis, J. Magn. Reson. 138 (1999) 199–209.
[21] C. Mailer, B.H. Robinson, B.B. Williams, H.J. Halpern, Spectral fitting: the extraction of crucial information from a spectrum and a spectral image, Magn. Reson. Med. 49 (2003) 1175–1180.
[22] R.P. Pandian, V.K. Kutala, N.L. Parinandi, J.L. Zweier, P. Kuppusamy, Measurement of oxygen consumption in mouse aortic endothelial cells using a microparticulate oximetry probe, Arch. Biochem. Biophys. 420 (2003) 169–175.
[23] G. Ilangovan, J.L. Zweier, P. Kuppusamy, Electrochemical preparation and EPR studies of lithium phthalocyanine—evaluation of the nucleation and growth mechanism and evidence for potential dependent phase formation, J. Phys. Chem. B104 (2000) 4047–4059.
[24] V.K. Kutala, N.L. Parinandi, R.P. Pandian, P. Kuppusamy, Simultaneous measurement of oxygenation in intracellular and extracellular compartments of lung microvascular endothelial cells, Antioxid. Redox Signal. 6 (2004) 597–603.