Parameter Estimation for Quantitative EPR Spectroscopy

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***Abstract*—Electron Paramagnetic Resonance (EPR) is a spectroscopy and imaging method based on detecting the spin resonance of electrons. EPR is particularly suited for detecting and quantifying free radicals and biological markers of oxidative stress, for which it is the only quantitative imaging method at the moment. Its application in clinical contexts is a major challenge because of the lack of standardized procedures for estimating the measurement quality. In our previous work, we conducted an analysis of the Lorentzian model of single-line EPR spectra in the derivative limit. For the general case, a modified acquisition procedure called over-modulation improves signal-to-noise ratio (SNR). In this manuscript, we study single-line EPR spectra with Lorentzian lineshape under over-modulation and propose Maximum Likelihood Estimates (MLE) as well as formulae for the expected relative error. We validate the results with simulated spectra and in vitro experimental results. The computed relative error is shown to be a good estimate of the observed instrumental variation.**

I. Introduction

General introduction

Electron Paramagnetic Resonance (EPR) has been well established as a viable technique for in vivo biomedical applications, such as dosimetry (measuring radiation dose) and oximetry (measuring tissue oxygen) [1]. EPR dosimetry focuses on retrospective estimation of irradiation dose based on the EPR signal amplitude [2]. In other words, EPR dosimetry utilizes the fact that the number of radiation-induced free radicals is proportional to the absorbed dose in solid parts of the body [3]. The applications of EPR in this field have been reviewed in several treatises both on the general technique [4]–[6] and on special subjects [7]–[12].

EPR oximetry, where the core problem is the estimation of the linewidth of the observed EPR spectrum, has emerged as a method of choice for a wide array of biological applications. Many publications have demonstrated that EPR measurements in living animals (in vivo EPR) can provide very significant new insights to physiology, pathophysiology and pharmacology [13]–[15]. While still at an early stage, oximetry measurements in human subjects have demonstrated the feasibility of the technique, including the capability of carrying out the measurements within the constraints of usual clinical practice [4], [16]–[18].

As of 2021, only six facilities worldwide are equipped to apply EPR in clinical settings. This requires a careful assessment of the EPR instrument capabilities, and of the precision of the measured signal amplitude and linewidth. While a few works have been previously published on improvements of the EPR hardware [19], [20], this paper focuses on the signal processing methods that are necessary to extract amplitude and linewidth from the raw EPR spectrum. In particular, we focus on estimating the precision based on a single spectrum, as large scale repeats are typically unavailable in clinical practice.

The lineshape itself and the various statistical parameters that can be derived from the lineshape (e.g. amplitude, linewidth, g-value) may contain much significant information, such as the quantum mechanic characteristics of the paramagnetic species or the linewidth and the amplitude of the signal which is relative to the paramagnetic species quantity and their surrounding. This paper focuses on linewidth and amplitude rather than other information.

The amplitude and the linewidth can be extracted from the EPR spectrum based on a model of the signal. Two commonly encountered lineshapes in various branches of molecular spectroscopy (including EPR spectroscopy) are the Gaussian and the Lorentzian. Consequently, the shapes of EPR lines are usually described by either Gaussian or Lorentzian functions.

For many interesting species, the absorption curve has only one peak and a bell shape. Therefore, the Gaussian function has been proposed as a lineshape model, and is well suited especially when the absorption curve is the superposition of many indistinguishable components [21]. For systems where linewidth is determined by electron spin relaxation (relaxation-determined), the recorded spectrum follows a Lorentzian function [22], [23]. In general, the linewidth is determined by the spin-lattice (T1), and spin-spin (T2) relaxation times. In this limit, the spin-lattice relaxation time (T1) does not affect the linewidth. See [24] for reviews of relaxation times.

When both types of contributions are appreciable, more complex functions have been proposed such as the Voigt profile [22], [25] or the Tsallis model [22], [26].

Parameter estimation is a well known method for extracting information from the model. There are two cases for linewidth and amplitude estimation: in vitro or for conditions where it is possible to have a good SNR, in such case one can set experimental parameters in the derivative limit (defined as modulation amplitude less than 0.1*linewidth [27], [28]); in vivo or in conditions where it is not possible to have a good SNR with a low modulation amplitude.

In the derivative limit case, methods for least-squares fitting of simulated spectra based on the spin Hamiltonian (sH) model to experimental spectrum have been largely developed. The

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main idea of these methods is estimating sH parameters, creating a simulated EPR spectrum fitting the experimental data. Many fitting methods in EPR had been reviewed in the book by Mabbs and Collison [29] and the book by Mirsa [30]. Stefan Stoll and Arthur Schweiger [31] developed Easyspin, a Matlab library to extract magnetic parameters from experimental EPR spectra by fitting a simulation to the experimental spectrum using least-squares fitting techniques. For lineshape, they proposed to use Lorentzian, Gaussian, and Voigt, and allow the operator to take modulation amplitude into account. Smirnov and Belford [32] devised a method based upon the Fourier transform to extract information from EPR spectra which have Voigtian lineshape containing a dispersive component. Bales [33], [34] analyzed the linewidth and the intensity of the first derivative EPR spectrum to deconvolve a Voigtian profile. Shin [35], [36] calculated Lorentzian-to-Gaussian linewidth ratio of a Voigtian profile using an analytical expression that contains the product of the linewidth and the maximum intensity.

In vivo EPR in the general case, using high modulation amplitude for magnetic field modulation (over-modulation) is a technique to get better signal [27]. Very few papers [37]–[39] described the effect of magnetic field modulation. They all used a Lorentzian lineshape model. Wahlquist [37] used a closed analytic expression to describe the modulation effects on a Lorentzian lineshape spectrum. Hyde et al. [38] proposed a filtering algorithm (pseudo-modulation) to simulate the effect of sinusoidal magnetic field modulation. Robinson et al. [39], [40] published a universal model to simulate the experimental EPR spectra in liquids by incorporating both the modulation amplitude and frequency. Based on Robinson's model, Mailer et al. [41] reported a remarkable precision improvement in the linewidth measurement of the deoxygenated OXO31 spin probe using EPR spectral-spatial imaging. Deng et al. [42] used curve fitting to extract linewidth information of LiNc-BuO as a function of oxygen concentration in spite of lineshape distortion. Palmer et al. [43] tried to find optimal values for magnetic field sweep and field modulation amplitude.

A neglected area is the analysis of the accuracy and precision of extracted parameters from a single experiment instead of a statistical analysis on a large number of replicate samples. This would drastically improve confidence in the observed values for in vivo and clinical EPR applications. Wahlquist [37] and Robinson [39] proposed the Lorentzian model, studied the properties of the model and its parameters (linewidth, modulation amplitude, etc.), without how to extract information from it. Smirnov and Belford [32] and Bales [33], [34] provided some information about the errors of the linewidth estimate of EPR spectra, but they did not discuss in detail how to get these information. Deng et al. [42] computed the standard deviation of the estimated parameter from numerous data, they could not show formal proof for that. In summary, error analysis with formal proof in the over-modulation case is missing.

In our previous paper [44], we addressed the derivative limit case and provided formulas for the extracting parameters and computing their standard deviation from individual EPR spectra. The derivative limit conditions can usually be met for in vitro experiments. However, poor SNR usually encountered in vivo usually requires setting the EPR instrument in the over-modulation regime. In this paper, we propose the MLE method to estimate parameters from over-modulated EPR spectra. In particular, we derive formulae for the precision of parameter estimates to evaluate our confidence in parameter extraction.

II. Theory

The EPR instrument measures how the sample absorbs microwave energy as a function of the magnetic field B in the cavity. This absorption curve $A[B]$ has several peaks in general that correspond to resonating conditions for the unpaired electrons, and are characteristic of the chemical compound. In this manuscript, we focus on single-line chemical species for which the Lorentz function has been proposed as a model for the absorption curve $A[B]$, and consider maximum-likelihood estimation of the model parameters.

A. EPR signal model

We consider the Lorentzian lineshape model for the absorption curve of single-line chemical species:

$$A[B] = \pi^{-1} \frac{\frac{1}{2}\text{FWHM}}{\left(\frac{1}{2}\text{FWHM}\right)^2 + (B - B_r)^2} \tag{1}$$

where FWHM is full width at half maximum of the noiseless absorption signal; B_r is the magnetic field value at resonance occurs. B_r is related to the g value of the paramagnetic molecule.

In most instrumental setups, the absorption curve is not observed directly. Instead, a field modulation technique with modulation amplitude MA is used and the EPR instrument measures finite differences contaminated by Gaussian noise:

$$X[B] = Cs(B) + \mathcal{N}(B) \tag{2}$$

$$s(B) = (A[B + \text{MA}] - A[B]) \tag{3}$$

where C is the area under the curve of the noiseless absorption signal, $N[B]$ is white Gaussian noise with standard deviation v (illustrated in Figure 1).

It is common practice to use small values of MA (called derivative limit), so that $s(B)$ can be approximated by $\text{MA}.A'[B]$ and the overall lineshape is a Lorentz derivative and independent of MA. In particular, FWHM can be obtained easily from the peak-to-peak distance in the derivative limit with the formula $\text{FWHM} = \sqrt{3}.\text{P2P}$ [22], [45]. However, in the general case, this lineshape appears distorted and current software tools do not perform parameter estimation adequately.

B. Maximum Likelihood Estimate

Maximum Likelihood is a standard statistical method for parameter estimation that is good when the statistical model is sufficiently regular. It consists in finding the set of parameters that maximizes the likelihood function:

$$\{\hat{\text{MA}}, \hat{C}, \hat{B}_r, \hat{\text{FWHM}}, \hat{v}\} = \arg\max \ln L \tag{4}$$

$$\ln L = -\frac{n}{2}\ln(2\pi v^2) - \frac{1}{2v^2}\sum_{k=1}^{n}\left(x(B_k) - C\, s(B_k)\right)^2 \tag{5}$$

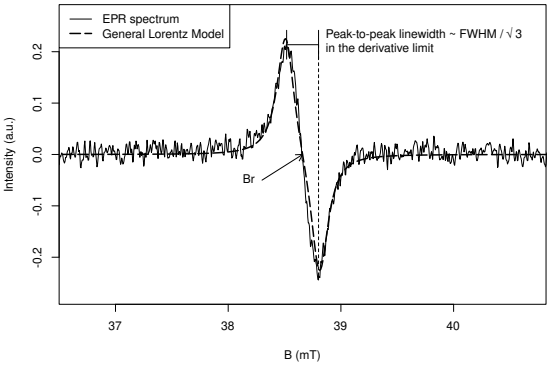

Fig. 1. Recorded single-line EPR spectrum with Lorentzian lineshape. Peak-to-peak linewidth (P2P) can be directly obtained from the EPR spectrum, but not Full Width Half Maximum (FWHM).

The parameter estimates are obtained with standard unconstrained nonlinear optimization methods. Initial estimates of the parameters can be computed with the following formulae:

$$\text{FWHM}_0 = |B_{\text{max}} - B_{\text{min}}| \tag{6}$$

$$C_0 = \frac{\max X(B)}{\max s(B)} \tag{7}$$

$$B_0 = \frac{|B_{\text{max}} + B_{\text{min}}|}{2} \tag{8}$$

$$v_0 = \frac{1}{n-1}\sum_{k=2}^{n}\frac{|X(B_k) - X(B_{k-1})|}{\sqrt{2}} \tag{9}$$

Where B_k for $i \in \{1, \ldots, n\}$ are the magnetic field values and B_{min} and B_{max} correspond to minimum and maximum intensity.

The EPR instrument provides a value for MA in the data files. However, we have encountered differences between the reported value and the MLE estimates, as well as temporal variations in practice. This has lead us to question the validity of the indicated value. Consequently, this manuscript is intended as theoretical foundations to further work on modulation amplitude in EPR, and we compute an MLE estimate $\hat{\text{MA}}$ based on the initial value provided by the instrument.

C. Precision of the parameter estimates

Due to Gaussian noise, parameter estimates obtained from an EPR spectrum are random variables, and we can compute lower bounds of their standard deviation from the Fisher Information Matrix (FIM) [46]. More precisely, starting from the Hessian H, we obtain :

$$H = \left[\frac{\partial^2 \ln L}{\partial^2(C, B_r, \text{FWHM}, \text{MA}, v)}\right] \tag{10}$$

$$\text{FIM} = -\mathbf{E}_x[H] \tag{11}$$

$$\text{cov}(\hat{\text{MA}}, \hat{C}, \hat{B_r}, \hat{\text{FWHM}}, \hat{v}) \sim \text{FIM}^{-1} \tag{12}$$

In practice, we use plugin estimates of the covariance matrix, and show that they match estimates obtained from repeated experiments in practice.

The following properties show that we only need to study the dependency of precision on MA and FWHM.

Proposition 1. *The estimation problem is translation-invariant with respect to B_r. Consider two EPR spectra corresponding to B_r^1 and B_r^2, and identical other parameters. Then the estimates verify :*

$$\hat{B_r^1} - \hat{B_r^2} = B_r^1 - B_r^2 \quad \textit{(i.e. same result up to translation)}$$
$$\theta_1 = \theta_2 \quad \textit{for the other parameters (FWHM, C, v, MA)}$$

Additionally, the standard deviations of the parameters estimates are the same for the two spectra.

Proof. The likelihood functions are translated versions of each other, and maximum-likelihood is attained at translated points. The covariance matrix is obtained through differentiation near the maximum. As the likelihood function shapes are the same, the covariances matrices coincide, and the precision of the parameter estimates are the same. □

Proposition 2. *The estimation problem is invariant with respect to C/v. Consider two EPR spectra corresponding to (C, v) and $(\lambda C, \lambda v)$ for $\lambda \in R$, and identical other parameters. Then the estimates are respectively $(\hat{C}, \hat{FWHM}, \hat{B_r}, \hat{v}, \hat{MA})$ and $(\lambda\hat{C}, \hat{FWHM}, \hat{B_r}, \lambda\hat{v}, \hat{MA})$. The covariance matrices are identical.*

Proof. $\ln L(\lambda C, \lambda v|x) = \ln L(C, v|x)$ □

Proposition 3. *The relative precision of the noise estimate $\hat{v}$ is $\frac{\sigma(\hat{v})}{v} = \frac{1}{\sqrt{n}}$.*

Proof. This is obtained when computing FIM^{-1} in (12) □

III. Applications

A. Simulated EPR spectra

We evaluate our parameter estimation method using simulated EPR spectra generated with Matlab from the Lorentzian lineshape model described earlier. As proved in Section II, B_r, C and noise intensity v have little effect on the behavior of the estimates, so we set $B_r = 39.142mT$ to match experimental values with the TAM probe in L-Band experiments, $C = 1000$ and $v = 10000$. Our simulations are set to explore different configurations of MA $\in \{0.01, 0.02, 0.04, 0.08\}$ mT and FWHM $\in \{0.01, 0.02, 0.04, 0.08\}$ mT and in particular the ratio MA/FWHM. We generated 1000 spectra for each of the 16 combinations.

B. Experimental Data

We performed an EPR analysis of Tetrathiatriarylmethyl tris - (8-carboxyl-2,2,6,6 - tetramethylbenzo - [1,2-d;4,5-d] bis[1,3] dithiol-4-yl) methyl sodium salt (TAM) to confirm our results on data acquired on an EPR spectrometer. TAM is a single line radical which has been developed for oxygen concentration determination by EPR. TAM have been proposed to selectively measure superoxide [46], [47]. It has the advantage to have a single narrow line with a very large signal-to-noise ratio which allows in vivo studies. The Lorentzian lineshape model is well adapted to model TAM EPR spectra [48].

TAM solutions were obtained according to [49]. TAM was solubilized in distilled water at 0.5 mM concentration, placed at room temperature, under atmospheric conditions in an EPR tube inserted in a 23 mm Bruker birdcage resonator mounted in a E540 Bruker EPR spectrometer. The data were collected on an L-band (1 GHz) CW EPR spectrometer under room-air conditions. Spectrometer parameters used were: Microwave power 2.005 mW, sweep width 1mT with 1024 points on the magnetic field abscissa, modulation frequency 10 kHz, sweep time 5.24s, Time constant and conversion time 5.12 ms. Under these conditions TAM EPR spectra should exhibit $B_r = 39.142$ mT and FWHM $= 0.025$ mT.

IV. Results

A. Simulated EPR spectra

For each simulated spectrum, we computed the MLE estimates and the precision using the MLE method described in II. As we have a series of 1000 spectra acquired in the same conditions on the same sample, we also compute the observed relative errors.

MA (mT)	FWHM (mT)	Predicted error (%)	Observed error (%)
0.01	0.01	0.15	0.16
0.02	**0.01**	**0.12**	**0.12**
0.04	0.01	0.13	0.13
0.08	0.01	0.15	0.15
0.01	0.02	0.84	0.84
0.02	0.02	0.44	0.43
0.04	**0.02**	**0.35**	**0.34**
0.08	0.02	0.36	0.38
0.01	0.04	8.32	8.56
0.02	0.04	2.39	2.33
0.04	0.04	1.24	1.24
0.08	**0.04**	**0.99**	**1.00**
0.01	0.08	264.68	76.20
0.02	0.08	25.62	34.56
0.04	0.08	6.72	6.80
0.08	0.08	3.51	3.62

TABLE I
C ESTIMATION ERRORS. MEAN PREDICTED ERROR COMPUTED FROM THE FIM, AND OBSERVED MEAN RELATIVE STANDARD DEVIATION BASED ON 1000 SPECTRA. BOLD VALUES INDICATE BEST PRECISION FOR EACH FWHM. THIS TABLE SHOWS THAT THE MLE ESTIMATE IS CORRECT (LOW ERRORS) AND THAT THE PREDICTED VALUES MATCH THE OBSERVED ERRORS. BEST RESULTS ARE OBTAINED FOR MA $=$ 2FWHM.

Table I and Table II show the predicted error computed from the FIM and the observed mean relative error of the MLE estimates $\hat{C}$ and $\hat{\text{FWHM}}$. First, we check that the MLE estimates are correct because the relative errors are small. Second, both tables show that the predicted relative errors match the mean observed relative error with a 1% margin for all combinations of MA and FWHM. Consequently, our predicted error faithfully reflects the amount of deviation that can be expected between the estimates and the true value based on simulations.

The performance of the estimates $\hat{C}$ and $\hat{\text{FWHM}}$ decreases with FWHM. This is because area-under-the-curve C and noise standard deviation v are fixed in these simulations. Consequently, higher values of FWHM correspond to more spread and lower maximum intensity for the absorption curve $A[B]$, and likewise for the finite difference $s(B) = (A[B + \text{MA}] - A[B])$. In conclusion, larger values of FWHM

MA (mT)	FWHM (mT)	Predicted error (%)	Observed error (%)
0.01	0.01	0.086	0.087
0.02	**0.01**	**0.082**	**0.083**
0.04	0.01	0.094	0.094
0.08	0.01	0.118	0.118
0.01	0.02	0.321	0.322
0.02	0.02	0.243	0.238
0.04	**0.02**	**0.234**	**0.231**
0.08	0.02	0.267	0.283
0.01	0.04	1.532	1.552
0.02	0.04	0.921	0.896
0.04	0.04	0.691	0.687
0.08	**0.04**	**0.663**	**0.672**
0.01	0.08	8.166	6.632
0.02	0.08	4.327	4.533
0.04	0.08	2.602	2.599
0.08	0.08	1.953	2.017

TABLE II
FWHM ESTIMATION ERRORS. PREDICTED ERROR COMPUTED FROM THE FIM, AND OBSERVED MEAN RELATIVE STANDARD DEVIATION BASED ON 1000 SPECTRA. BOLD VALUES INDICATE BEST PRECISION FOR EACH FWHM. THIS TABLE SHOWS THAT THE MLE ESTIMATE IS CORRECT (LOW ERRORS) AND THAT THE PREDICTED VALUES MATCH THE OBSERVED ERRORS. BEST RESULTS ARE OBTAINED FOR MA $=$ 2FWHM.

means that the EPR signal decreases with respect to noise. This is relevant in practice, but linewidth is a chemical characteristic of the compound, and not a parameter that can be set during the experiment.

Contrary to linewidth, modulation amplitude MA can be selected during the experiment. Table I and Table II suggest that MA should be set such that the ratio MA/FWHM ~ 2 for best estimate precision. This is different from current recommendations which are to set the ratio MA/FWHM $= 1$ in over-modulation experiments [43] for best Signal-to-Noise ratio. Table III presents mean peak-to-peak intensity of the 1000 EPR spectra for the same combinations of MA and FWHM. This table confirms that best intensity (and thus best SNR) is attained for MA/FWHM $= 1$.

MA (mT)	FWHM (mT)	Intensity (a.u)
0.01	**0.01**	**9.94E+06**
0.02	0.01	9.06E+06
0.04	0.01	7.18E+06
0.08	0.01	5.40E+06
0.01	0.02	2.19E+06
0.02	**0.02**	**2.49E+06**
0.04	0.02	2.28E+06
0.08	0.02	1.82E+06
0.01	0.04	3.74E+05
0.02	0.04	5.61E+05
0.04	**0.04**	**6.28E+05**
0.08	0.04	5.67E+05
0.01	0.08	0.66E+05
0.02	0.08	1.03E+05
0.04	0.08	1.59E+05
0.08	**0.08**	**1.71E+05**

TABLE III
MEAN PEAK-TO-PEAK SIGNAL INTENSITY OF 1000 SPECTRA AS A FUNCTION OF MA AND FWHM. BOLD VALUES INDICATE BEST INTENSITY FOR EACH VALUE OF FWHM, AND THUS BEST SNR. THIS TABLE SHOWS THAT BEST INTENSITY IS REACHED FOR MA/FWHM $= 1$.

B. Experimental Data

One TAM sample was prepared as in III. By varying the value of modulation amplitude MA $\in \{0.02, 0.04, 0.08\}$ mT, we obtain three different series of 1000 spectra.

Instrument MA	0.02 mT	0.04 mT	0.08 mT
Estimated $\hat{C}$	0.00220	0.00224	0.00245
Predicted precision	0.0000285	0.0000221	0.0000267
Observed precision	0.0000335	0.0000236	0.0000181
Estimated $\hat{\text{FWHM}}$ (mT)	0.0252	0.0254	0.0275
Predicted precision	0.000161	0.000157	0.000210
Observed precision	0.000172	0.000154	0.000131
Estimated $\hat{\text{MA}}$ (mT)	0.0190	0.0375	0.0696
Predicted precision	0.0000600	0.0000482	0.0000678
Observed precision	0.0001152	0.0000994	0.0000844

TABLE IV
ESTIMATED VALUES OF $\hat{C}$, $\hat{\text{FWHM}}$, $\hat{\text{MA}}$ AS A FUNCTION OF THE MA VALUE SET IN THE INSTRUMENT, AND THEIR MEAN PREDICTED PRECISION AND MEAN OBSERVED PRECISION ON 1000 EPR SPECTRA. OUR PREDICTIONS ARE IN GOOD AGREEMENT WITH THE OBSERVED ERRORS ON IN VITRO EXPERIMENTS.

In Table IV we study parameter estimates of $\hat{C}$, $\hat{\text{FWHM}}$, $\hat{\text{MA}}$ as a function of the MA value set in the instrument for the experimental dataset. We show the estimated value (average over 1000 spectra), the predicted precision (average over 1000 spectra) and the average observed precision.

There is no theoretical value for $\hat{C}$, so we can only expect that the estimates are independent of MA because C only related to the sample in this model. The observations support this. The estimates $\hat{B}_r$ and $\hat{\text{FWHM}}$ match the expected values of $B_r = 39.142$ mT (not shown) and FWHM $= 0.025$ mT, and are stable. The estimate $\hat{\text{MA}}$ matches the value set in the instrument, although it seems underestimated, which may explain overestimation of FWHM for MA $= 0.08$ mT.

Overall, the predicted precision matches the observed precision. As the predicted precision only accounts for signal processing and disregards other sources of variation, we can expect the observed precision to be higher. Table IV shows that the computed precision is good enough to be usable in practice. The situation is similar for the other parameters estimates (data not shown).

V. DISCUSSION

In most EPR experiments, the ratio MA/FWHM is set lower than 1/10. This avoids inhomogeneous linewidth broadening [29]–[36] which makes direct linewidth measurements not accurate [22], [27], [45]. Consequently, the finite difference $s(B) = (A[B + \text{MA}] - A[B])$ can be approximated by the derivative $s(B) \sim \text{MA}.A'[B]$. This is called the derivative limit, and we provided an MLE estimate for this setting in [44]. An advantage of using the derivative limit is that it emphasizes rapidly changing features of the EPR spectrum [23].

In the derivative limit, FWHM can be directly measured for single line EPR spectra from the distance between the maximum and minimum intensity, i.e. the peak-to-peak distance. In the Lorentzian lineshape model, the FWHM can be computed from the peak-to-peak linewidth according to FWHM $= \sqrt{3}$.P2P [22], [45]. In the general case, the relationship is more complex, and the observed peak-to-peak distance is a combination of linewidth and modulation amplitude (see [27] for more detail). Consequently, P2P values obtained from experiments performed with different values of MA cannot be easily compared.

Similarly, area-under-the-curve for the absorption function A is used to measure the total number of spins in the sample, and relate to concentration. For EPR spectra, double integration is needed to recover AUC, but this is also dependent on modulation amplitude because $s(B) \sim \text{MA}.A'[B]$. Consequently, we believe that C and FWHM are better parameter choices for EPR experiments than double integral or peak-to-peak distance and will better reflect the chemical properties of the sample.

In our experience, the derivative limit works well when SNR is higher than 20dB, with precision on the order of 1% (see [44]). However, SNR is typically poorer for in vivo experiments, and overmodulation is used as a signal amplification technique.

In [42], [43], the authors point out that using MA equal to 0.5 times FWHM or larger can yield better EPR spectra, but at the cost of distorting the derivative lineshape. In this manuscript, we propose a method that explicitly takes into account this distortion and we show that it is applicable for in vitro experiments. Additionally, the MLE approach provides estimates of the precision without the need to perform repeat experiments.

As reported in [37], [43], maximum peak-to-peak intensity is obtained for MA = FWHM, as in Table III. This translates to maximum peak signal-to-noise ratio, but not necessarily to best estimation quality. According to our experiments, optimal conditions for estimating FWHM linewidth and amplitude C are closer to MA $=$ 2.FWHM. This is partially supported by the experimental dataset in Table IV. Predicted precision is best for all parameters for MA $= 0.04$ mT, but observed precision is decreasing. The exact reason for this is not clear, but may be related to the difference between the MA value set in the instrument and the estimated value $\hat{\text{MA}}$. In particular, optimal conditions are obtained for overmodulated EPR spectra.

The accuracy of linewidth measurements directly translates to oxygen measurements. The linewidth of soluble molecules such as TAM is known to increase with the oxygen concentration. For most chemicals, the linewidth is 10 mT larger at 21% oxygen compared to 0% oxygen [46]. In our TAM dataset, the predicted errors in linewidth correspond to roughly 1% in oxygen concentration. Additionally, it has been shown that the relationship is linear with respect to peak-to-peak linewidth in derivative limit conditions [50]. This study should enable us to measure oxygen in more difficult experimental conditions thanks to overmodulation.

The results presented in this manuscript are only applicable to EPR spectra matching the Lorentzian lineshape model, which is often the case for liquid samples [22], [39]). For other models such as Gaussian or Voigt lineshapes, the MLE approach can be applied, but the exact formulas need to be recomputed.

VI. PERSPECTIVES AND CONCLUSION

We demonstrated that the MLE method allows accurate determination of the five fundamental parameters of an EPR spectrum in the Lorentzian lineshape model (C, B_r, FWHM,

MA, and noise intensity v). Contrary to classical methods (such as Easyspin), we provided estimates of the measurement quality, which is critical for clinical applications (e.g. in measuring the oxygen level of biological tissues). The provided formulae correspond to the Lorentzian model for the EPR signal under the effects of magnetic field modulation and are applicable for systems that the linewidth are relaxation-determined (such as liquid). Our results need to be extended to the other case when EPR samples which do not follow the Lorentz model.

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