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Spectral Modeling for Accelerated pH Spectroscopy using EPR

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Abstract

A data modeling and processing method for electron paramagnetic resonance (EPR)-based pH spectroscopy is presented. The proposed method models the EPR spectrum of a pH-sensitive probe in both protonated and unprotonated forms. Under slow-exchange conditions, the EPR spectrum of a sample with an unknown pH value can be accurately represented by a weighted sum of the two models, with the pH value completely determined by their relative weights. Unlike traditional pH spectroscopy, which relies on locating resonance peaks, the proposed modeling-based approach utilizes the information from the entire scan and hence leads to more accurate estimation of pH for a given acquisition time. By employing the proposed methodology, we expect a reduction in the pH estimation error by more than a factor of three, which represents an order of magnitude reduction in acquisition time compared to the traditional method.

Keywords

EPR; pH; acidosis; modeling; nitroxide; spectroscopy

1. Introduction

Perturbation in pH homeostasis is associated with several pathological states, including renal disease [1], inflammation [2], ischemia [3], chronic lung disorder [4], intrauterine abnormalities [5], and stomach ulceration [6]. Also, certain stress conditions—such as biochemical shock, interruption of normal blood supply, and high exercise levels—may suppress the body's ability to self-regulate local pH values [7, 8]. More importantly, local pH values may interfere with delivery, absorption, and pharmacological effectiveness of drugs, and with efficacy of different treatment regimens [9, 10]. For example, in tumors, extracellular pH is lower than in normal tissue and can be correlated with the disease progression, prognosis, and response to radiotherapy and chemotherapy [11, 12]. Therefore, accurate measurement of region-specific pH values using noninvasive methods carries considerable biomedical and clinical relevance.

Electron paramagnetic resonance (EPR) is a branch of spectroscopy in which electrons with unpaired spins, when placed in a magnetic field, absorb electromagnetic radiation to transition from a low-energy level to a high-energy level. Environment-dependent spectral changes of paramagnetic materials have led to widespread biological application of EPR,

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including measurement of oxygen [13], pH [14], perfusion [15], redox metabolism [16], and detection of short-lived radicals [17].

Measurement of pH using EPR relies on quantifiable and reversible changes in the spectra of exogenous pH probes [18]. The imidazoline and imidazolidine nitroxide radicals (NRs) are the most commonly used pH-sensitive EPR probes [19]. Figure 1 shows 4-amino-2,2,5,5-tetramethyl-3-imidazoline-1-yloxy (ATI) [20], a commonly used imidazolidine NR. The protonation of the N-3 atom of ATI leads to a decrease in unpaired electron density at the N-1 atom and is manifested in the EPR spectrum as a decrease in the hyperfine coupling constant.

Figure 2 shows the EPR spectrum of ATI, with three resonance peaks, measured using a continuous-wave (CW) L-band (1.2 GHz) EPR spectrometer at two different pH values, one on either side of pK_a . The sample at pH = 4.00 is primarily comprised of the protonated form (RH^+), while the sample at pH = 8.30 is primarily comprised of the unprotonated form (R) of the NR. A noticeable difference between the spectra of RH^+ and R is the relative location of the resonance peaks. Traditionally, pH is inferred from one of two spectral characteristics: either location of a resonance peak with respect to a fixed reference point or the separation between two resonance peaks. In either case, the quantity measured from spectrum is compared to a calibration curve to yield a pH estimate.

In CW EPR, where the first field-modulation harmonic of the absorption spectrum is observed, the locations of resonance peaks are generally identified by measuring the zero-crossings of the spectrum, as shown in Fig. 2. In some studies, peak locations in the integrated spectrum have been used in lieu of zero-crossings [14]. In general, the location of the highest-field peak displays the greatest difference between R and RH^+ , and hence is the most sensitive indicator of pH.

EPR is inherently more sensitive than nuclear magnetic resonance due to the larger magnetic moment of an electron compared to that of a proton. EPR is also superior in terms of functional specificity due to the absence of overlapping EPR signals from endogenous species. Recent development of low-frequency CW and pulsed EPR systems [21, 22] along with development of sensitive, stable, and nontoxic pH probes [23, 24], has broadened the applicability of EPR-based techniques. In fact, this technique has already been used to measure the stomach pH in live murine subjects [25], monitor pH of tumor tissue in live mice [26], characterize release processes in biodegradable polymers [27], and study the influence of a drug on the microacidity of skin in humans and rats [28].

Despite recent efforts, EPR data acquisition for pH spectroscopy and imaging remains a time-consuming proposition, especially when high measurement accuracy is desired. For *in vivo* applications, where conditions may change rapidly, it is desirable to accelerate the acquisition process of pH spectroscopy without sacrificing measurement accuracy. A major shortcoming of the conventional EPR-based pH measurement method, called hyperfine coupling method (HCM), is its reliance on a small fragment, e.g., zero-crossing, of the noisy measured spectrum. In this work, we propose and verify a modeling-based approach, called parametric modeling method (PMM), that utilizes the information from the entire measured spectrum to improve the measurement accuracy.

The remainder of the paper is organized as follows: Section 2 describes parametric modeling of EPR spectra and the related pH estimation procedure; Section 3 discusses material and methods for L-band spectroscopy experiments; Section 4 presents results; Section 5 includes discussion; and Section 6 summarizes the conclusions.

2. Spectral modeling and pH estimation

For a probe with known pK_a value, pH is defined by

$$p = pK_a - \log_{10} \left(\frac{\alpha}{\beta} \right), \quad (1)$$

where α and β define the concentrations of RH^+ and R , respectively, and p represents the pH value.

In the case of slow-exchange, any measured spectrum with arbitrary pH value can be accurately represented by a superposition of RH^+ and R spectra [29]. Once the RH^+ and R spectra have been modeled, the relative contributions (α/β) of RH^+ and R spectra, for a given sample with unknown pH, can be estimated by fitting the measured spectrum with a weighted sum of the two modeled spectra. The pH value can then be inferred using Eq. 1. In this section, we describe the parametric modeling of RH^+ and R spectra; we then present an estimator for the unknown pH value.

2.1. Spectral modeling

The first step is to parameterize the EPR spectrum of a pH-sensitive EPR probe. To ensure the adequacy of a small magnetic field sweep, the parametric modeling of the spectrum has been restricted to the highest-field resonance peak. Also, we use multiple Voigt functions to describe the spectra. The use of multiple functions provides a more accurate representation of complex spectra, e.g., ones with ^{13}C satellite peaks. Using multiple Voigt functions, a parameterized spectrum for RH^+ , denoted by $I^+(B)$, can be expressed as

$$I^+(B) = \sum_{k=1}^K \rho_k^+ \underbrace{\mathcal{L}(B, \Gamma^+) * \mathcal{G}(B, B_k^+, \sigma_k^+)}_{\text{Voigt}}, \quad (2)$$

where K represents the total number of Voigt functions used in the modeling; ρ_k^+ represents the intensity of the k^{th} Voigt function; $\mathcal{L}$ stands for a first-derivative Lorentzian function; B represents the external magnetic field; Γ^+ denotes half-width at half-maximum linewidth of $\mathcal{L}$; $\mathcal{G}$ represents a Gaussian function centered at B_k^+ with standard deviation σ_k^+ ; and $*$ represents a convolution in the first variable.

In CW EPR, signal-to-noise ratio (SNR) can be improved by applying overmodulation [30]. To incorporate overmodulation in our modeling, we replace the first derivative Lorentzian function, $\mathcal{L}$, with the first harmonic of an overmodulated Lorentzian function, $\tilde{\mathcal{L}}$, defined by Robinson et al. [31], yielding

$$I^+(B) = \sum_{k=1}^K \rho_k^+ \underbrace{\tilde{\mathcal{L}}(B, B_m, \Gamma^+) * \mathcal{G}(B, B_k^+, \sigma_k^+)}_{\text{Overmodulated Voigt}}, \quad (3)$$

where B_m is the field modulation amplitude.

Likewise, the spectra of R can be modeled using a combination of over-modulated Voigt functions to generate $I^-(B)$, yielding

$$I^-(B) = \sum_{k=1}^K \rho_k^- \underbrace{\tilde{\mathcal{L}}(B, B_m, \Gamma^-) * \mathcal{G}(B, B_k^-, \sigma_k^-)}_{\text{Overmodulated Voigt}}. \quad (4)$$

For a given probe, $I^+(B)$ and $I^-(B)$ are constructed by fitting measured spectra of RH^+ and R with Eqs. 3 and 4, respectively, and estimating the parameters accented with '+' and '-' superscripts on the right sides of Eqs. 3 and 4.

2.2. pH estimation

Once both $I^+(B)$ and $I^-(B)$ have been separately constructed, a spectrum, $I(B)$, with an arbitrary pH value, under the assumption of slow-exchange [29], can be written as

$$I(B) = \alpha I^+(B) + \beta I^-(B). \quad (5)$$

For a measured noisy spectrum, $Y(B)$, the values of α and β can be estimated using least-squares fitting, yielding

$$(\hat{\alpha}, \hat{\beta}) = \arg \min_{\alpha, \beta} \|Y - I\|_2^2, \quad (6)$$

where Y and I are vector representations of sampled spectra $Y(B)$ and $I(B)$, respectively. The unknown pH can be computed from $\hat{\alpha}$ and $\hat{\beta}$ using Eq. 1.

3. Material and Methods

In this section, we describe the procedures used in preparing pH-sensitive samples. We also briefly mention the protocols used for EPR data collection.

3.1. Sample preparation

The sample was prepared by dissolving 1 mM ATI in 10 mM phosphate buffer solution. A total of ten ATI samples, with pH values ranging from 4.00 to 8.30, were prepared. The pH was adjusted by adding 0.1 M HCl and NaOH solutions to the sample. After the pH was adjusted and measured using a pH meter, a small volume (0.25 ml) of each sample was poured into a separate 0.5 ml Eppendorf PCR tube. Figure 3 shows the pH values of all ten samples.

The s^+ and s^- samples, with pH values more than two units away from pK_a , were assumed to be comprised of purely protonated and unprotonated forms and were used to build $I^+(B)$ and $I^-(B)$, respectively. The other eight samples, $s_1, s_2, \dots, s_8$, were used for validation. After the preparation and before the EPR measurements, the samples were stored in a refrigerator at 4°C.

3.2. Data collection

The data were collected on an L-band CW EPR spectrometer under room-air conditions. The spectrometer was fitted with automatic tuning and coupling mechanisms and an 8 mm diameter surface-coil resonator to hold the samples. Two datasets were collected for each sample: one at low modulation amplitude (0.036 mT) and one at high modulation amplitude (0.18 mT). Only the highest-field peak was scanned. Each dataset had ten scans collected under identical conditions. The other acquisition parameters were: magnetic field sweep

width=1.6 mT, center of magnetic field sweep=48.78 mT, acquisition time per scan=8 s, microwave power=0.5 mW, and number of data samples per scan=1024.

4. Results

In this section, we verify the proposed method using experimental data. Also, we draw a comparison between PMM and HCM in terms of measurement accuracy or, equivalently, data acquisition time.

The parametric models, $I^+(B)$ and $I^-(B)$, were constructed from the measured spectra of s^+ and s^- samples, respectively. First, all ten scans from s^+ and s^- were independently averaged to improve SNR, and the averaged spectra were then modeled using Eqs. 3 and 4 to generate $I^+(B)$ and $I^-(B)$, respectively. The process of constructing $I^+(B)$ and $I^-(B)$ was repeated for both low-modulation and high-modulation datasets.

Three overmodulated Voigt functions ($K=3$ in Eqs. 3 and 4) were used to describe the spectra for both the datasets. One Voigt function was used to describe the visibly dominant resonance peak, while the other two Voigt functions were used to describe the small ^{13}C satellite peaks that reside on either side of the dominant peak. The amplitudes (ρ) of the ^{13}C satellite peaks were only 3% to 5% of the dominant peak. For the low-modulation dataset, peak-to-peak linewidths (ΔB_{pp}) of $I^+(B)$ and $I^-(B)$ were observed to be 0.1024 mT and 0.0878 mT, respectively. For the high-modulation dataset, ΔB_{pp} values of $I^+(B)$ and $I^-(B)$ were observed to be 0.1585 mT and 0.1537 mT, respectively. Figure 4 shows the modeling results for both the datasets.

For each dataset, measurements from $s_1, s_2, \dots, s_8$ as well as from s^+ and s^- were fitted with a weighted sum of $I^+(B)$ and $I^-(B)$ to estimate α and β and hence the pH values. Figures 5 and 6 show examples of fitting results for the s_3, s_5 , and s_7 samples. Figures 7 and 8 display the mean and standard deviation of pH values estimated using PMM and HCM. Table 1 compares the average standard deviation of estimated pH for the two methods for $s_1, s_2, \dots, s_8$ samples.

The calibration curves, T_{HCM} and T_{PMM} , shown in Figs. 7 and 8 are defined as,

$$\begin{aligned} T_{PMM}(p) &\equiv \frac{1}{1+10^{(c_l-p)}}, \\ T_{HCM}(p) &\equiv \frac{c_d}{1+10^{c_s(c_k-p)}+c_d}, \end{aligned} \quad (7)$$

where the parameters c_l and c_k represent the pK_a value of the probe and the other parameters (c_a, c_d and c_s) define the shape of the sigmoid function.

For T_{HCM} , all four parameters were estimated by fitting the T_{HCM} function to the observed h vs. p data, with h being the measured location of the highest-field resonance peak from an arbitrarily selected fixed reference point. Traditionally, the sigmoid function for T_{HCM} has been defined in terms of three parameters, with the fourth parameter, c_s , fixed at one. The motivation behind including c_s as an additional parameter is to generate a tight fit between T_{HCM} and h vs. p curve for a wide range of B_m . The shape of h vs. p curve changes significantly with B_m , with smaller B_m generating larger growth rates and thus requiring larger values of c_s . For low-modulation dataset (Fig. 7), the values of c_a, c_d, c_k , and c_s were estimated to be 76.9 μT , 1.7 μT , 6.241, and 1.785, respectively. For high-modulation dataset (Fig. 8), the values of c_a, c_d, c_k , and c_s were estimated to be 78.3 μT , 0.85 μT , 6.296, and 1.276, respectively. After estimating all four parameters of the T_{HCM} function, the pH values were determined by

$$\tilde{p} = c_k - \frac{1}{c_s} \log_{10} \left(\frac{c_a}{h - c_d} - 1 \right), \quad (8)$$

where $\tilde{p}$ is the pH estimate based on HCM.

For PMM, the value of c_l was determined by fitting T_{PMM} function to $\hat{\beta}/(\hat{\alpha} + \hat{\beta})$ vs. p data, with $\hat{\alpha}$ and $\hat{\beta}$ determined using Eq. 6. The estimated values of c_l were found to be 6.289 and 6.291 for the low-modulation and high-modulation datasets, respectively. Once the value of c_l was estimated, the pH values of the samples were calculated from the measured data by

$$\hat{p} = c_l - \log_{10} \left(\frac{\hat{\alpha}}{\hat{\beta}} \right), \quad (9)$$

where $\hat{p}$ is the pH estimate based on PMM.

5. Discussion

EPR-based pH spectroscopy relies on reversible changes in the EPR spectrum as a function of pH. The conventional method, HCM, quantifies the unknown pH value by comparing the location of a zero-crossing of one of the resonance peaks against a previously determined calibration curve. Although, this approach has been extensively used for pH measurements, it is inefficient as only a small number of data points of the measured spectrum are actually utilized in calculating pH. The proposed modeling-based approach, PMM, on the other hand, models the entire resonance peak (or peaks) and hence utilizes all the measured spectrum for computing pH.

In PMM, the complexity of the model (Eqs. 3 and 4) in itself does not impact the accuracy of the method as long as the spectra are accurately modeled. For ATI, a combination of three overmodulated Voigt functions was adequate to accurately represent spectra of RH^+ and R at two different modulation amplitudes. Using more than three Voigt functions to model I^+ (B) and I^- (B) spectra (results not shown) yielded no improvement in the pH measurement accuracy. Other NRs, with more complex spectra, may require more than three Voigt functions for accurate modeling. On the other hand, the spectral differences between RH^+ and R can impact the accuracy of both HCM and PMM significantly. For example, a probe with large hyperfine coupling differences between the spectra of RH^+ and R is more likely to produce accurate results as opposed to a probe with small hyperfine coupling differences.

For HCM, it is a common practice to use a sigmoid function to approximate observed variations in hyperfine coupling as a function of pH. However, depending on the spectral shape of the probe and the experimental parameters, the approximation may not be reasonable as there is no physical reason for the observed hyperfine coupling to follow a sigmoid function exactly. For example, if EPR spectra of R and RH^+ exhibit different linewidths, which is generally the case, the h vs. p curve no longer possesses the odd-symmetry of a sigmoid function. Therefore, for such cases, estimating pH using T_{HCM} may lead to systematic errors in the estimation of pK_a . In fact, for HCM, we observed a B_m dependent error in the estimation of pK_a . For the low-modulation dataset, HCM underestimated pK_a by approximately 0.06 units. When the lowest-field peak was considered (data not shown) instead of the highest-field peak, HCM underestimated pK_a by approximately 0.1 units for the low-modulation dataset. The trend of underestimating pK_a even held for the traditional calibration curve, which entails fixing the value of c_s to one. This B_m dependence of estimated pK_a can be attributed to asymmetries originating from the

lineshape differences between R and RH^+ , which are more pronounced at smaller B_m values as evident from the mismatch between ΔB_{pp} values of $I^+(B)$ and $I^-(B)$. The previously reported pK_a of ATI, when measured at X-band using HCM, is 6.1. It is likely that this reported pK_a value is an underestimation of the true value for the reasons discussed here. For the high-modulation dataset, the pK_a values determined by the two methods, HCM and PMM, were in close agreement.

The $\beta/(\alpha + \beta)$ vs. p curve by definition follows the sigmoid function of T_{PMM} , enabling PMM to estimate pK_a more accurately and without any systematic bias. The pK_a values reported by PMM were consistent, within 6.90 ± 0.005 , for both the modulation amplitudes and for both the highest-field and lowest-field peaks (data not shown).

The proposed method is based on the assumption of slow-exchange, which ensures that any spectrum can be modeled as a weighted sum of $I^+(B)$ and $I^-(B)$. It has been previously demonstrated by Khramtsov et al. and others that the assumption of slow-exchange can be ensured by limiting concentrations of NR and accompanying buffer as well as by selecting $3 < pK_a < 11$ [32, 33, 19]. Generally, concentrations of 1 mM or less for NR and 10 mM or less for buffer are adequate to ensure slow-exchange [34, 35].

The structure in the residual, especially for the s_5 sample with $p \approx pK_a$ (Fig. 5), is probably due to a deviation from the slow-exchange assumption. This small deviation, however, does not exhibit significant impact on the pH estimation. The accuracy of results (Figs. 7 and 8), which explicitly rely on the slow-exchange assumption, provides validation of the assumption for the reported experimental conditions. Note the extracellular buffer concentrations encountered *in vivo* are even lower than what we have used in our experiments. Therefore, by limiting the concentration of ATI to 1 mM, we can ensure slow-exchange to be a reasonable assumption under *in vivo* conditions. For a given NR, *ex vivo* calibration studies, similar to one presented here, should be conducted to establish the conditions required for slow-exchange, including limits on the concentrations of NR and buffer.

In this work, we have assumed that s^+ and s^- consist of purely RH^+ and R , respectively. This is a reasonable assumption as s^+ and s^- contained less than 1% contamination from R and RH^+ , respectively. If spectra corresponding to sufficiently high and low pH values are unavailable, any other pair of spectra with sufficiently different pH values can be used instead as long as the precise pH values of the samples are accurately known. For example, spectra from s_3 ($p = 5.70$) and s_7 ($p = 6.71$) can be modeled using Eqs. 3 and 4 and later used to fit the spectrum from a sample with unknown pH. However, using samples with pH values close to pK_a may require more than three Voigt functions for accurate modeling (see Fig. 5).

Traditionally, multiple resonance peaks are scanned, and the estimation of pH is based on the separation between two peaks. Scanning multiple peaks suppresses the impact of unintended spectral shifts due to excitation frequency drift that may arise due to animal motion. However, this approach can lead to longer acquisition times due to wider scans. Since we are proposing scanning a single resonance peak, the collected data must be corrected for a frequency drift. Although we did not observe any frequency drift for the spectroscopy experiments, a drift, if present, must be accounted for to generate accurate, reproducible results. We suggest using either of two procedures to tackle the issue of frequency drift during *in vivo* measurements: (i) Use wider scans to build $I^+(B)$ and $I^-(B)$ and record the corresponding excitation frequency using a frequency counter. For a given modulation amplitude and pH-sensitive probe, this step needs to be performed only once under *ex vivo* conditions. During *in vivo* data acquisition, record the excitation frequency

immediately before or after each scan. The sweep width of *in vivo* scans should be smaller than the sweep width used for $I^+(B)$ and $I^-(B)$ scans. For *in vivo* scans, compute shifts in measured spectra, in the units of magnetic field, based on the frequency difference of each scan with respect to $I^+(B)$ and $I^-(B)$ scans. Before fitting each scan using Eq. 5, digitally shift $I^+(B)$ and $I^-(B)$ to match the observed shift in the scan and then truncate the shifted $I^+(B)$ and $I^-(B)$ to match the narrower sweep width of the scan. (ii) Record the excitation frequency for scans used to build $I^+(B)$ and $I^-(B)$. Design a hardware feedback loop that on-the-fly changes the center of sweep width in proportion to the frequency drift from the previously recorded value for $I^+(B)$ and $I^-(B)$ scans. Measure and process the *in vivo* data as if there were no frequency drift. Also, a small uncertainty in the location of the resonance peak, if present despite the proposed measures, can be tolerated by including it into the model as we have previously demonstrated for oximetry application [36].

Figures 7 and 8 display the experimental results against the ideal calibration curves defined in Eq. 7. As reported in Table 1, PMM exhibits high estimation accuracy for both the low-modulation and high-modulation datasets. For the low-modulation dataset, PMM provides sixfold improvement while for the high-modulation dataset, PMM provides over threefold improvement compared to HCM. Since the standard deviations of $\hat{\rho}$ or $\tilde{\rho}$ are proportional to SNR, which, in turn, is proportional to the square-root of scan time, the data acquisition speedup offered by PMM is a factor of 36 for the low-modulation dataset and a factor of 13 for the high-modulation dataset.

Under high SNR conditions, the measured spectra of RH^+ and R can be effectively used in lieu of $I^+(B)$ and $I^-(B)$, respectively. A parametric modeling of RH^+ and R spectra, however, carries two major advantages. First, parametric modeling of EPR spectra would allow optimization of acquisition parameters, including field modulation amplitude and sweep width, via statistical sensitivity analysis [37]. Second, the parametric modeling would enable developing forward models, which relate the EPR projection data to spatial distribution of α and β , and thus potentially reducing the data acquisition time for pH imaging. A similar concept has already been applied to EPR imaging of oxygen, providing a factor of forty reduction in the acquisition time. [38].

6. Conclusions

We have presented a data modeling and processing approach to reduce the acquisition time of EPR-based pH spectroscopy by over an order of magnitude. The method is based on parametric modeling of the EPR spectrum and relies on slow-exchange, which is a reasonable assumption for the experimental setup adopted in this work and is expected to hold valid under *in vivo* conditions. The method is verified using measurements from a commonly used pH-sensitive probe for a physiologically relevant pH range.

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- A data modeling and processing method for EPR-based pH spectroscopy is presented.
- The method is based on parametric modeling of EPR spectra under slow-exchange conditions.
- The proposed method offers an order of magnitude reduction in acquisition time.
- For validation, L-band spectroscopy studies are reported.

Figure 1. Protonation of 4-amino-2,2,5,5-tetramethyl-3-imidazoline-1-yl-oxyl (ATI), an imidazoline nitroxide radical commonly used for EPR-based pH measurements. The pK_a value of ATI has been reported to be 6.1.

Figure 2.

Three-peak EPR spectrum of ATI collected at two different pH values using a CW L-band spectrometer. The spectra were collected with 0.036 mT field modulation amplitude and 5.01 mT field sweep. The vertical lines provide a visual comparison of the peak locations. Here, 'o' represents zero-crossings of the resonance peaks.

Figure 3.

A total of ten ATI samples, each with a different pH value, were prepared. A small volume (0.25 ml) of each sample was placed in a separate 0.5 ml Eppendorf PCR tube. The samples with the lowest pH value, s^+ , and the highest pH value, s^- , were used to construct parametric models $f^+(B)$ and $f^-(B)$, respectively, while the other eight samples, $s_1, s_2, \dots, s_8$, with intermediate pH values were used for validation.

Figure 4.

Spectral modeling of the highest-field resonance peak of ATI sample at two extreme pH values. The modeling of s^+ spectrum with $p = 4.00$ yielded $l^+(B)$, while the modeling of s^- spectrum with $p = 8.30$ yielded $l^-(B)$. The modeling was performed using Eqs. 3 and 4 for two different field modulation amplitudes: 0.036 mT (a) and 0.18 mT (b).

Figure 5.

Fitting results of the low-modulation (0.036 mT) dataset. Three scans—one for s_3 , one for s_5 , and one for s_7 —are depicted (a). The spectra were fitted with a weighted sum of previously constructed $l^+(B)$ and $l^-(B)$ shown in Fig. 4a. The resulting components, $l^+(B)$ and $l^-(B)$, are also shown (b).

Figure 6.
The fitting process shown in Fig. 5 is repeated for high-modulation (0.18 mT) dataset.

Figure 7.

Estimated pH values plotted against the calibration curves (solid gray line) for low-modulation dataset for both HMC (a) and PMM (b). Error bar, for each sample, indicates ± 5 standard deviations of estimated pH values. Here, h represents the separation between measured zero-crossing of the highest-field resonance peak and a fixed reference point, which in this case was arbitrarily chosen at 48.74 mT.

Figure 8.
Estimation of pH values, similar to the one shown in Fig. 7, for the high-modulation dataset.

Table 1

pH measurement accuracy of traditional HCM and proposed PMM. Here, $\overline{std}$ indicates the standard deviation of the estimated pH values averaged across the eight samples, $s_1, s_2, \dots, s_8$.

	HCM ($\bar{p}$)	PMM ($\bar{p}$)
low-modulation dataset ($B_m = 0.036$ mT)	$\overline{std}=6.53 \times 10^{-2}$	$\overline{std}=1.08 \times 10^{-2}$
high-modulation dataset ($B_m = 0.180$ mT)	$\overline{std}=2.23 \times 10^{-2}$	$\overline{std}=0.61 \times 10^{-2}$