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RESEARCH ARTICLE

Lock-in Amplifier Dairy Sensor for Detection of Ciprofloxacin

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ABSTRACT This work introduces a sensor for dairy antibiotic detection of ciprofloxacin in milk. We provide an important tool for antibiotic detection in milk and the main contributions of this work are as follows. We introduce a technique based on florescence spectroscopy and lock-in amplification for sensing ciprofloxacin in milk that is capable of detection below the regulatory limit. We compare the system against one without the integrated lock-in amplification, as in traditional fluorescence sensors. We provide microchip capillary electrophoresis results and place our work in context for future integration with microchip capillary electrophoresis.

INDEX TERMS Microfluidics, biosensors, dairy products, amplifiers, bio-MEMS.

I. INTRODUCTION

Antibiotics are used prevalently in the global dairy industry for disease prevention, disease treatment, and stimulating feeding efficiency [1], [2]. Consequently, antibiotics can threaten human health by developing antibiotic resistant strains of bacteria and inducing allergic reactions when antibiotic compounds are consumed through dairy products [2], [3], [4]. Currently used antibiotic biosensors are manual and can be affected by human error. A modern challenge is rapid on-site detection of antibiotic contamination in dairy parlours and related dairy applications [5], [6], [7].

Previous studies have used microchip capillary electrophoresis (MCE) for dairy antibiotic detection. This MCE technique is a miniaturized and versatile technology which can be adopted for dairy applications [8], [9], [10], [11], [12], [13]. Here, electric fields can move and separate analytes. Specifically, as the sample particles migrate, the sample will separate into its constituent particles at different rates based on their charge-mass ratios, allowing constituents to be isolated and easily detected due to different travel times [14], [15], [16].

Fluorescence spectroscopy (FS) is fundamental to microfluidic systems including MCE operation. Fluorescence spectroscopy, in isolation, will detect a single fluorescent analyte such as one antibiotic. In the case of livestock, such as dairy cows, one antibiotic compound is often administered to treat a specific ailment [17], [18]. The implementation of MCE for single antibiotic detection of ciprofloxacin through FS has been utilized [11], [19] as the antibiotic ciprofloxacin emits fluorescence at 440 nm when excited by a light source of 280 nm [20]. The fluorescence based dairy MCE system designed by Bosma et al. [11] yielded a detection limit of 61.1 mg/kg of milk, whereas the regulated limit of ciprofloxacin in bovine milk is 0.1 mg/kg of milk [21]. The described sensitivity limitations of MCE systems are a product of a signal-to-noise ratio (SNR) in need of improvement.

A common detection system used for antibiotic residues is the Delvotest® T [6], [22]. This best detects tetracyclines and ß-lactams. This system works by measuring an inhibitory substance within a sample of milk. The detected inhibitory substance is the antibiotic residue above its limit-of-detection

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(LOD). The Delvotest® T requires manual operation. The Charm® Rapid One-Step Assay (ROSA®) [5], [23] requires incubation of a milk sample mixed with buffer solution, within the test strip. Gold-bead receptors are used in a lateral-flow format for detection and measurement of tetracycline and ß-lactam antibiotics within a milk sample. The Charm® ROSA® test also requires well-trained personal to make qualitative decisions in the manual processes. Therefore, automated and easy-to-implement solutions, such as FS sensors, are desirable for on-farm implementation.

To globally implement any dairy sensors, be they MCE systems or stand-alone FS systems, the LOD must be improved as the sensitivity of previously developed dairy antibiotic detection systems utilizing FS is inadequate [24].

This work explores a solution to improve the LOD of FS through the integration of a lock-in amplifier (LIA). Due to its sensitive detection capabilities, LIAs have been widely used in various applications [25], [26], [27], [28], [29], and miniaturized into portable systems [30], [31], [32]. Additionally, LIA systems are used in ultrafast optics [33], [34], [35], [36] and terahertz spectroscopy [37], [38], [39], [40] photonic systems.

This LIA dairy application comes about through advancements from our prior work, which is summarized here. In Bosma et al. [19] we present preliminary results of a MCE system, with no integrated lock-in or other electronic processing of the results. This preliminary result showed promise for future development of microfluidic devices for the dairy industry. In Bosma et al. [11], we further develop the MCE device for detection of antibiotics in milk. The LOD is drastically higher (61.1 mg/kg) than the regulatory limit, indicating a pronounced need for lowering of the LOD. In Bosma et al. [12], we present a detailed analysis of the electronics required for MCE, specifically focusing on development of our custom voltage sequencer for pinched sample injection and its properties. In Eswar et al. [41] we present the actuation and logistical methods for using a digital microfluidic platform (i.e., droplet-based microfluidics utilizing electrowetting-on-dielectric EWOD) for actuation of dairy milk, but do not explore sensing of antibiotics. In Eswar et al. [42], we explore a basic digital microfluidic device for application to dairy testing. This digital microfluidic system includes a lock-in amplifier in its signal-flow diagram. However, there is no discussion and analysis of a comparison to a DC system of signal collection. There is no discussion of future integration with MCE systems. Furthermore, due to the confines of the digital microfluidic structure, with associated hydrophobic and dielectric layers, this digital microfluidic device is limited to only achieve a LOD above the regulatory limit, further indicating a strong need for an exploration of a dedicated lock-in dairy sensor.

There is a great opportunity to apply LIA in a dairy sensor, and lower the LOD to the regulatory limit, and this is the focus of our work. The main contributions of this work are as follows. First, we introduce a technique based on FS and LIA for sensing ciprofloxacin in milk that is capable of detection below the regulatory limit. Second, we compare a baseline system against our device. The baseline system is without the integrated lock-in amplification, as in traditional fluorescence sensors. Third, we provide MCE results and place our work in context for future integration with MCE.

The manuscript is organized as follows. Section II provides fundamental knowledge of lock-in amplification, and Section III shows our equipment setup and operation procedures. The signals and noise levels are analyzed in Section IV, and the LOD and limit-of-quantification (LOQ) are analyzed in Section V. Section VI discusses other techniques and compares their attributes. Section VII discusses future integration with MCE systems. Section VIII discusses anti-interference, and concluding remarks are made in Section IX.

II. FUNDAMENTALS OF LOCK-IN AMPLIFICATION

Fundamentally, lock-in amplification detects periodically modulated signals at a reference frequency determined by the user. The LIA can detect signals at the reference frequency with a narrow bandwidth. In contrast, typical high performance electrical filters possess a wide bandwidth [43]. For appropriate use of a LIA, the desired signal must be modulated at a reference frequency before encountering noise interference.

For optical experiments such as FS, modulating the light source prior to any interaction with the sample or afterwards is crucial. Modulating the signal at the emission source prevents noise from being modulated at the same reference frequency. If the noise is modulated at the same reference frequency as the desired signal, the LIA will amplify the noise as well as the desired signal. In this work, modulation of the desired signal is induced by optically chopping the excitation light source using a digital function generator. A digital function generator is beneficial for modulation as it can connect its output frequency to the reference input of the LIA. Connectivity between the modulation source and LIA ensures that the reference signal generated is at the same frequency and phase as the emission source. The output of the LIA is a DC signal proportional to the components at the reference frequency. We will discuss the behavior of a LIA, including results from detecting ciprofloxacin with and without a LIA.

III. EQUIPMENT AND OPERATION

To test the effectiveness of noise reduction using lock-in amplification, FS sensor tests are performed on milk samples injected with ciprofloxacin at multiple concentrations. The milk is filtered from fat using a 0.45 μm pore filter before adding ciprofloxacin. The 1 mM stock solution of ciprofloxacin in milk is diluted with additional milk for tests at multiple concentrations of ciprofloxacin.

The photodiode used is the DET36A2 biased Silicon (Si) Photodiode (Thorlabs, New Jersey, USA). The UV LED used is the LTPL-G35UV275GC-E (LITE-ON Technology, Taipei, Taiwan). This UV LED requires a forward voltage of 7.2 V. The optical filter used to isolate the fluorescent

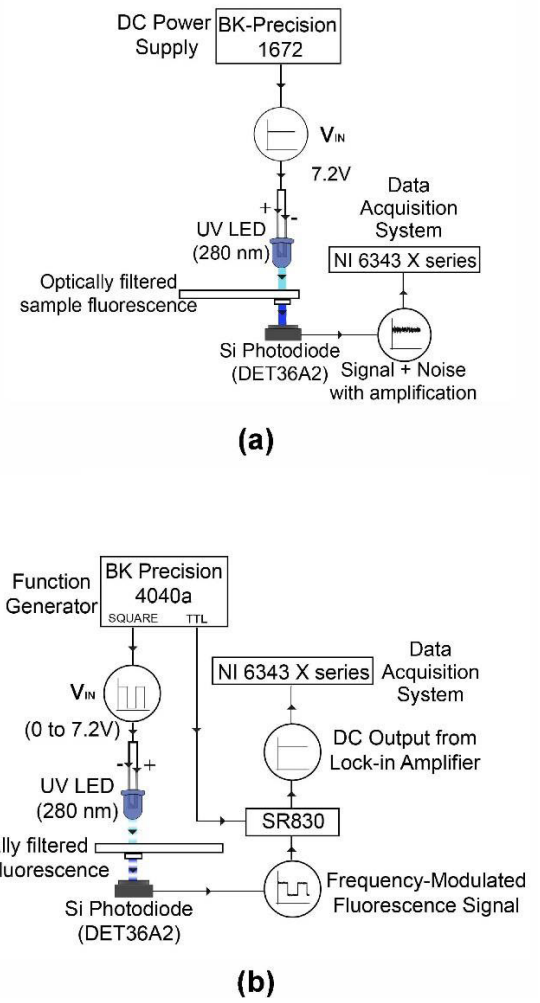

FIGURE 1. Shown here is a schematic of equipment to perform the SNR tests of the antibiotic detection system with a 100 μL sample of 0.8 mM of ciprofloxacin in milk, where (a) the excitation UV LED is powered by a DC power supply. (b) The excitation UV LED is powered by a function generator supplying a sinusoidal forward voltage for emission and reference frequency for the LIA output.

transmission (440 nm) is the Edmund Optics 86-339 (New Jersey, USA). The lock-in amplifier is the Stanford Research Systems SR830 (California, USA).

The photodiode and UV LED are mounted onto XYZ translational stages to align the UV LED directly over the active area of the photodiode. A glass slide holding the milk sample is then held in between the UV LED and photodiode to perform the tests.

When performing the tests without lock-in amplification, the input signal to the LED is a 7.2 V DC signal from the BK-Precision 1672 (California, USA), as seen in Fig. 1(a). When lock-in amplification is implemented, the LED is powered using the BK Precision 4040a function generator, which generates a square wave signal, ranging from 0 to 7.2 V at a reference frequency of 2.5 kHz, as seen in Fig. 1(b). The reference frequency is chosen far from the DC noise range ($<$ 100 Hz) to reject the system and environmental noise.

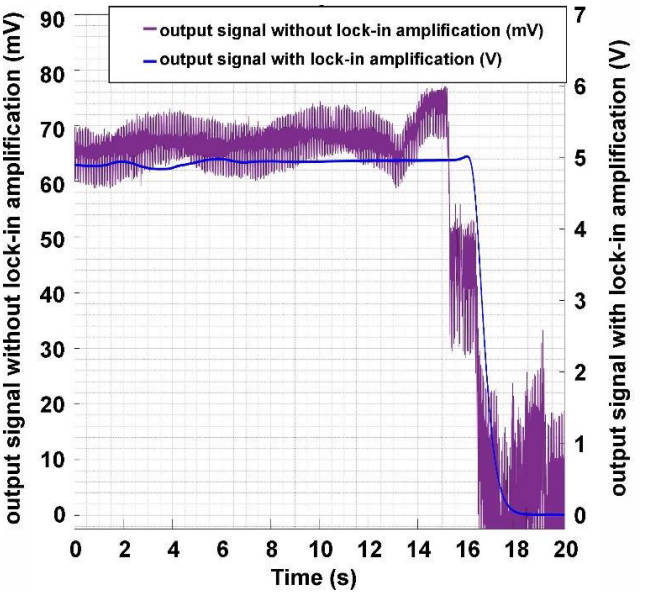

FIGURE 2. The data is collected and plotted for both experimental conditions, where the purple curve and left axis represent the fluorescence signal without lock-in amplification, and the blue curve and right axis represent the fluorescence signal with lock-in amplification. The disturbance between 15/16 seconds of the DC signal is attributed to the signal's transition between the photodiode's detection of a fluorescence signal and the baseline noise of the system.

The TTL output of the function generator is connected to the SR830 LIA, ensuring the LIA detects signals at the reference frequency.

The parameters used on the LIA are as follows: sensitivity of 1 mV, time constant of 1 s with filter slope of 24 dB/oct, and high dynamic reserve. With a sensitivity of 1 mV, the gain is 10,000 [43]. The time constant of 1 s with a 24 dB/oct roll-off entails the cut-off frequency of the lowpass filter is 1 Hz. The slope of 24 dB/oct is the steepest slope available on the LIA. A steep slope with a time constant of 1 s allows the LIA to pass frequencies below 0.1 Hz, while frequencies above 3 Hz are fully attenuated. The high dynamic reserve indicates that a high level of noise can be tolerated on the input circuitry of the LIA.

Shown in Fig. 2 is data collected using lock-in amplification, overlayed with a trial without lock-in amplification, for tests with 100 μL samples of 0.8 mM of ciprofloxacin in milk. The results show an apparent difference in output signal quality (i.e., voltage magnitude) with and without the LIA, emphasizing its importance in optical experiments. The recorded signal without lock-in amplification shows that there is a difference between baseline and fluorescence, however, the periodicity of the signal without lock-in amplification shown in Fig. 2 highlights the high level of noise and interference which is otherwise rejected in the case of lock-in amplification. The detection portion of the LIA system measured and demonstrated here utilized samples of milk to explore the fundamental optical detection, which is similar to that used in MCE.

IV. SIGNAL AND NOISE ANALYSIS

Noise is a common error in optical applications. Optimal biosensor performances require low noise levels, i.e., a high SNR. A low SNR can obstruct desired signals, especially if the signal of interest is small. A method used in optical systems to improve detection of small signals is lock-in amplification. Inherently, a LIA is a sensitive bandpass filter and amplifier.

The most controllable noise sources are extrinsic noise sources, which encompass external light sources, capacitive coupling, and inductive coupling. To demonstrate the noise rejection capabilities of the LIA, external light sources were not eliminated for all FS detection experiments conducted in this work. Without LIA, the detected signal is expected to be noisy as it will detect light at all modulated frequencies. On the contrary, the output signal of the LIA is expected to possess less noise from external light sources. To minimize capacitive coupling noise corresponding to voltage measurements, the signals were measured using low impedance BNC cables, and physically separating the cables far apart. Reduction of inductive coupling related noise was not necessary as no current measurements were made.

The SNR in this work is quantified as a unitless ratio as

$$SNR = \frac{V_{RMS:fl} - V_{RMS:base}}{V_{noise}}. \quad (1)$$

The root-mean-square (RMS) voltage ($V_{\mathrm{RMS:base}}$) is the measured voltage of the photodiode when there is no ciprofloxacin present (i.e., ''blank sample'') in between the photodiode and UV LED. The voltage measurement when the milk sample is present corresponds to ($V_{\mathrm{RMS:fl}}$). The denominator (V_{noise}) of (1) represents the quantification of the background noise within the FS system. The noise is quantified as the standard deviations of ($V_{\mathrm{RMS:fl}}$) and ($V_{\mathrm{RMS:base}}$). The noise is relatively consistent between ($V_{\mathrm{RMS:fl}}$) and ($V_{\mathrm{RMS:base}}$), hence an average of the noise within the two regions is used for all computations. This definition of SNR is consistent with that of Galievsky et al. [44].

Error corresponding to a concentration of ciprofloxacin is determined by computing the standard deviation of all trials at the corresponding ciprofloxacin concentration. The number of trials ranges from three to six trials per concentration, for concentrations ranging from 3.1 μM to 1 mM of ciprofloxacin in milk. The fluorescence response of the DC system is shown in Fig. 3(a). Based on the DC response curve, there is a general positive relationship between concentration of ciprofloxacin in milk and the output voltage. At the highest concentration, the signal is 107 mV. This is a detectable signal by most modern electronics. However, the standard error is quite high within each concentration measurement, impeding the ability to accurately distinguish between different ciprofloxacin concentration levels. The inability to distinguish between concentrations would be a characteristic of a low sensitivity system. The ability to distinguish between concentrations would be a characteristic of a high sensitivity system. The sensitivity is defined as the slope of the line of best fit, corresponding to 103 mV/mM for experimental trials without a LIA.

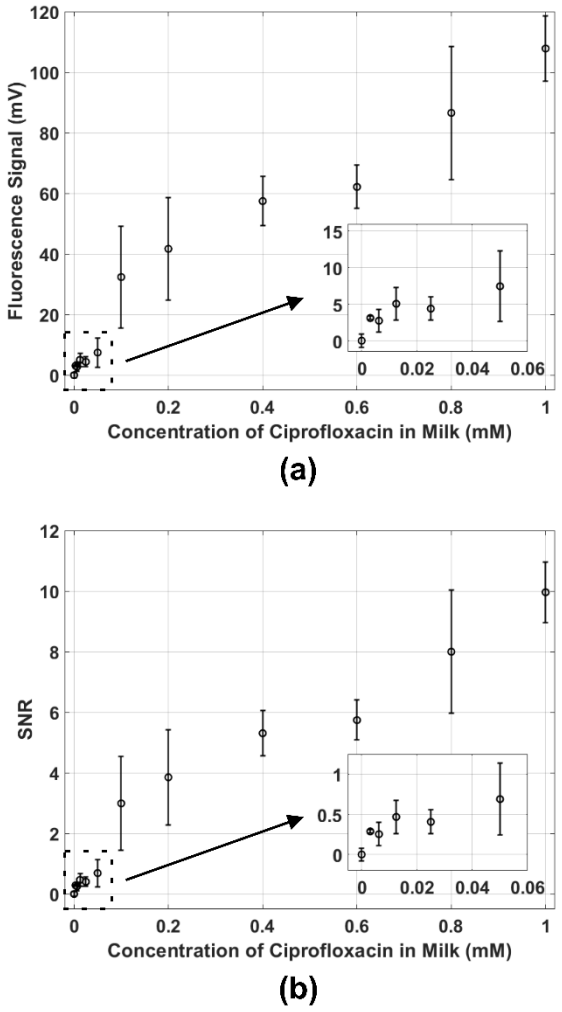

FIGURE 3. (a) The fluorescence signal at various concentrations of ciprofloxacin in milk, and (b) the corresponding SNR without lock-in amplification. A linear model is used to fit the data. This results in an R^2 value of 0.95 for the system without LIA. The insets highlight the values corresponding to concentration of 0-0.05 mM where the lowest concentration of ciprofloxacin used was 3.1×10^{-3} mM. The error bars represent the standard deviation of three trials, with the circle marker representing the mean.

According to the work conducted by Galievsky et al. [45], the SNR for a FS detection system can be compared despite the measured signal having differing bandwidths due to varying choices of a bandpass filter. The bandwidth of the measured signal does not inhibit the ability to compare the SNR between systems, it is the measurement device (i.e., system bandwidth) that dictates the ability to compare the SNR between the LIA and DC measurements. Both the LIA and DC measurements use the same sensor device (i.e., photodiode) for the fluorescence spectroscopy measurements—ensuring the same system bandwidths. The same bandpass filter (Edmund Optics 86-339) was used for the LIA and DC systems presented in this work, ensuring that the measured

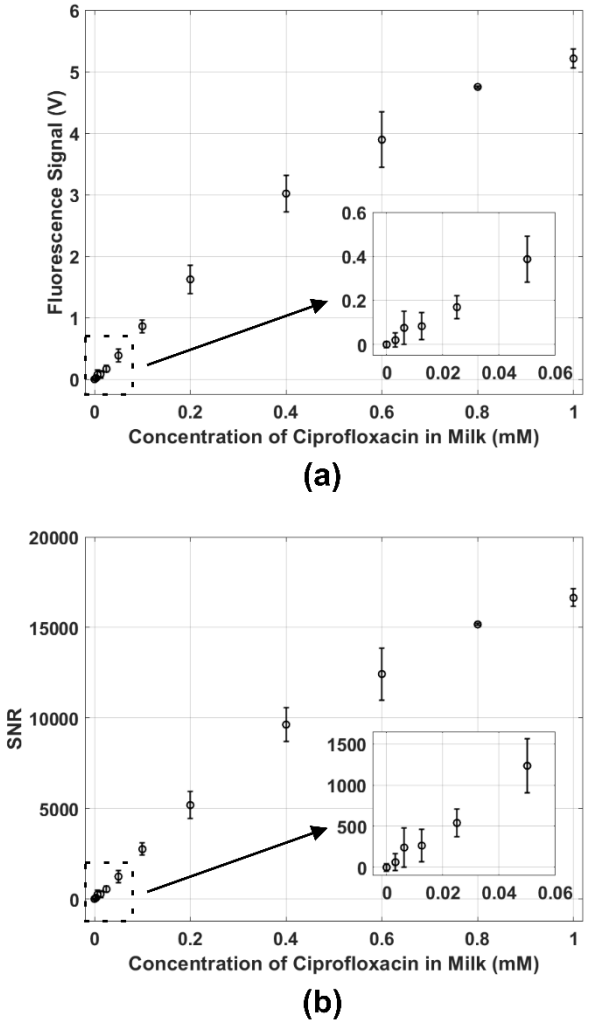

FIGURE 4. (a) The fluorescence signals and (b) corresponding SNR of the FS detection system with lock-in amplification implemented. A linear model is used to fit the data. This results in an R^2 value of 0.98 for the LIA system. The insets highlight the values corresponding to concentration of 0-0.05 mM where the lowest concentration of ciprofloxacin used was 3.1×10^{-3} mM. The error bars represent the standard deviation of three trials, with the circle marker representing the mean.

signal bandwidths by the photodiode are identical providing additional rigor to the SNR comparison. The SNR curve of the detection system without lock-in amplification is shown in Fig. 3(b). Referring to the vertical axis of Fig. 3(b), it is evident that the overall SNR is low, as it reaches a maximum of approximately 10. The experiment is repeated with the same equipment and environment, but with the implementation of lock-in amplification. The fluorescence signal amplitudes using LIA are shown in Fig. 4(a). The sensitivity of 5.61 V/mM emphasizes that implementing a LIA greatly improves the FS detection system. The peak optical power used in both the DC and LIA detection method is 10 mW with 7.2 V applied.

To further demonstrate the detection enhancement of lock-in amplification, the SNR of the fluorescence signals with LIA is shown in Fig. 4(b). When comparing the SNR range to that of Fig. 3(b), the magnitude of the SNR is much higher, reaching a maximum of approximately 17,000. The lock-in amplification enhances the SNR of the FS detection system by approximately three orders of magnitude. Improving both the measured peak and SNR of the FS detection scheme corresponds to an improvement to the LOD, discussed in the following section.

In Fig. 4(b), there is an observable slope change resulting in an overall sublinear trend of the Fig. 4 data, which can be perceived as a regime for 0-0.4 mM with a slope of approximately 8 V/mM and a regime for 0.4-1 mM with a slope of approximately 4 V/mM. One such cause for this behavior would be saturation of the detected signal that occurs on the photodetector. Such an interpretation would yield a higher quotation of the slope of the response curve, and thus an even lower LOD. However, we choose the more conservative quotation of the slope (and therefore LOD) and note that it is within the regulatory limit.

V. ANALYSIS OF THE LIMIT-OF-DETECTION AND LIMIT-OF-QUANTIFICATION

In this section, the experimental results and analysis of SNR will be discussed in the context of the LOD. The LOD is calculated from the method reported by Guider et al. [46] that is, calculated as three times the standard deviation of the noise divided by the sensitivity, for 99% certainty. The equation used to compute the LOD is

$$LOD = \frac{3\sigma}{m}, \tag{2}$$

where σ is the uncertainty, or noise level of the biosensor, and m is the sensitivity (i.e., slope) of the response curve. Sensitivity is the system's ability to measure a change in quantity. In the context of MCE, sensitivity describes how well the measurement system can discriminate finite amounts of analyte. Literature shows that a highly sensitive detection system is one that can measure low LOD values which supports (2) [45]. In the presented work, a high m value (i.e., high sensitivity) is obtained with the lock-in amplification detection.

The results corresponding to FS antibiotic detection without lock-in amplification emphasize that the FS optical detection scheme is an adequate technology to detect ciprofloxacin in milk [47]. The LOQ is

$$LOQ = \frac{10\sigma}{m}, \tag{3}$$

However, FS alone exhibits minimal electronic filtering, achieving the low SNR values depicted in Fig. 3(b), which corresponds to the inability to detect the regulated limit of ciprofloxacin in milk. Upon integration of a LIA, the SNR values depicted in Fig. 4(b) corresponds to an improvement to the sensitivity of ciprofloxacin for the FS detection system. To improve the LOD in fluorescence-based MCE, it is necessary to improve the SNR. Improving the SNR can be achieved by amplifying the measured signal and/or reducing noise.

TABLE 1. Summary of limits of detection and quantification.

System	Limit-of-detection (mg/kg)	Limit-of-quantification (mg/kg)
Traditional (DC) Electronics FS	114	380
MCE with DC Excitation [11]	61.1	204
Regulatory limit [21]	1.00×10^{-1}	
LIA Electronics FS [this work]	5.20×10^{-2}	1.73×10^{-1}

Therefore, to make a system more sensitive, is to improve the system's LOD, which corresponds to an improvement to the SNR [48], [49].

An amplifier that proportionally increases the signal and noise will not improve the SNR, and hence no improvement to the sensitivity or LOD will coincide. An amplifier that amplifies the signal but not the noise will still improve the SNR, and the LOD will improve along with the sensitivity. An amplification system that amplifies the measured signal and reduces noise, such as a LIA, improves the SNR of the system which correspondingly improves the LOD and sensitivity of the system [48], [49], [50], [51].

Limits of detection were computed for the FS detection system with and without a LIA. Ultimately, these will be compared to the LOD of the system developed by Bosma et al. [11] which uses a DC excitation source for the MCE. The LODs are converted from mM of ciprofloxacin in milk to mg of ciprofloxacin per kg of milk (mg/kg), knowing that the molar mass of ciprofloxacin is 332 g/mol [52], and the density of milk is 1030.8 g/L [53]. A summary of all the LODs measured are tabulated in Table 1, along with the regulated maximum residual limit (MRL) of ciprofloxacin in milk [21]. The DC system has a poor LOD of 61.1 mg/kg. However, the implementation of lock-in amplification greatly improves the LOD for ciprofloxacin in milk. Since LIA electronics has a steeper slope m than both DC systems, the LIA technique is a more sensitive system. The LOD calculation for the FS test with lock-in amplification in this study results in a low LOD of 0.052 mg/kg. This is approximately half the regulated limit of ciprofloxacin in milk (0.1 mg/kg) [21]. LOD_{DC}/LOD_{LIA} describes the improvement factor between the LIA and DC (electronic FS) measurements. The presented data yields an improvement factor of 2200. The implementation of LIA results in a large improvement for SNR and LOD. The improved metrics associated with LIA validate the implementation of lock-in detection for dairy antibiotic detection. The concentration of LODs in units of mM are 0.353 mM without LIA, and 1.61×10^{-4} mM with LIA.

VI. OTHER TECHNIQUES

Our work can be discussed in the context of other (laboratory) commercial techniques, including liquid chromatography-mass spectroscopy (LC-MS), gas chromatography-mass spectrometry (GC-MS), and capillary electrophoresis (CE).

The LC-MS technique can achieve a LOD of approximately 0.001 mg/ml [54]. While this is an impressive LOD, the large device size (approximately 2 m^3) of LC-MS makes it cumbersome and does not lend itself to use on farm, thus it is a lab-based technique. Trained operators are required and device cost can be prohibitive.

The GC-MS technique can achieve a similar LOD [55], however, it is also large and cumbersome and is largely a lab-based technique that is ill-suited to on-farm applications. Trained operators are required to collect and interpret data.

The capillary CE technique can achieve impressive LOD values, e.g., 5 μg/mL [56], however, its size limits its ability to be applied on-farm. Here also, trained personnel are required.

In contrast, our dairy sensor is small and compact, relatively simple, and has great potential for on-farm applications. It has an acceptable LOD relative to the regulatory limit, and therefore it is a viable option for on-farm implementation.

VII. FUTURE INTEGRATION WITH MICROCHIP CAPILLARY ELECTROPHORESIS

The time for detection that we found in our experiments was less than five minutes. This is the approximate time that cattle spend in the milking parlor [57], and therefore we believe our technique to be adequate in terms of the time for detection. For future integration with MCE, whereby analyte separation can occur, the problem of long detection time can also be discussed. Detection time can be lessened by using a smaller microfluidic chip with a shorter separation channel, while maintaining the same electric voltages [9], [10]. This would increase the electric field over the distance, thus increasing the charges on the constituent particles, increasing the travel velocity of the particles. This can be achieved through strategic MCE designs. The length of the separation channel must still be carefully designed to ensure that the antibiotics are completely separated from other constituents to create a well-resolved electrophoretic peak. For MCE, another way to reduce analysis time would be to increase the applied voltages, which would also result in an increased electric field. The disadvantage here would be an increase of joule heating in the microfluidic channels and wells. Joule heating would occur due to an increased current in the microcapillaries, which can lead to peak dispersion, reducing the separation resolution, and possibly increase fouling within the microfluidic channels. Consideration for future integration with MCE is further discussed below.

To ensure the channels of the MCE system are cleaned after every use, a solution of 1.0 M NaOH is used. The NaOH solution also promotes deprotonation of the silica molecules on the microfluidic channel walls to enhance the electroosmotic mobility of the microfluidic channels. Flowing throughout the channels of the MCE system is a buffer solution. The actuation and control of the flow direction of the buffer solution is pivotal to isolate or "pinch" a milk sample for efficient segregation by electrophoresis. Literature shows that

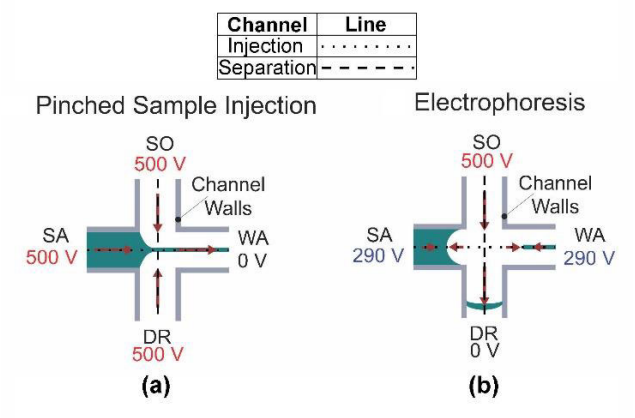

FIGURE 5. Seen here are the voltages applied to the source well (SO), sample well (SA), waste well (WA), and drain well (DR) of the microfluidic chip to carry out (a) pinched sample injection and (b) electrophoresis, where the milk sample is represented by the blue fluid and buffer solution is represented by the white fluid.

a solution of 0.1 M citrate is an effective buffer between the pH ranges of 3.0 to 6.0 [58] and can be adjusted to a pH of 5.5 using sodium hydroxide [59].

The solution of 0.1 M citrate buffer is prepared by mixing 1.2 g of sodium citrate dihydrate, 1.1 g of citric acid, and 80 mL of ultrapure water. Once the pH is corrected, ultrapure water is added until the buffer reaches a volume of 100 mL. Ciprofloxacin HCl Monohydrate purchased from LKT Laboratories (Minnesota, USA) is mixed with the filtered milk. A stock solution of 1 mM of ciprofloxacin in milk is mixed, and sequentially diluted for tests at multiple concentrations. Additionally, milk samples are filtered using a 0.45 μm pore filter to remove fat components which would accumulate along the microfluidic channels. After filling the microfluidic channels with buffer solution, the sample well of the microfluidic chip is drained using a pipette and loaded with 1.35 μL of milk.

To carry out fluid actuation and separation for detection of antibiotics in milk samples, pinched sample injection is performed [60]. Pinched sample injection ensures that only a minute volume of a sample is delivered to the detection region, ensuring a distinguishable onset and termination of the electrophoretic peak during detection. Detecting a well-defined peak is advantageous as it ensures all elements within the milk sample are fully detected, as opposed to only detecting the components of the leading edge of a continuous sample stream. Pinched sample injection is feasible with MCE by controlling the voltages actuated upon the milk and buffer solution using a lab-built voltage sequencer developed by Bosma et al. [12]. This voltage sequencer is a network of NPN and PNP transistors designed to source a single high voltage input from a high voltage power supply and supply four independent voltages from 0 V to the supplied voltage. A depiction of the voltage configurations of the MCE system for the pinched electrophoretic conditions are shown in Fig. 5.

To control and actuate fluid flow conditions, voltages are controlled at four microfluidic wells. The milk is injected into the sample well, while the buffer solution is injected in the source and drain well. The waste well acts as a receptacle to capture fluid flown into it. The 10 mm long channel connecting the sample and waste wells is known as the injection channel. The 85 mm long channel from the source to the drain well is known as the separation channel. The FS detection scheme is located 12.5 mm before the drain well. For the pinched sample injection configuration of Fig. 5(a), a high voltage of 500 V is applied to the sample, source, and drain wells, while the waste well is grounded. In this configuration, the milk sample will flow towards the waste well in tandem with the buffer solution from the source and drain wells. The buffer flowing to the waste well from both sides of the milk sample creates a thin consistent or ''pinched'' stream of milk flowing across the intersection of the injection and separation channels [10].

To separate the pinched milk sample from the sample well and actuate a minute volume of it to flow towards the FS detection scheme, electrophoresis is utilized. To actuate a milk sample to undergo electrophoresis, a voltage of 500 V is applied to the source well, a lower voltage of 290 V is applied to the sample and waste wells, and the drain well is grounded. A graphical representation of the electrophoresis process is shown in Fig. 5(b). The lower voltage of 290 V—and equivalent electric field—is applied to the sample and waste wells to actuate buffer solution flowing from the source, to flow into the sample and waste wells. The buffer solution flowing into the waste and sample wells prevent milk from flowing into the drain well as a continuous stream of milk. The value of 290 V was chosen iteratively by adjusting the voltage between trials until the electrophoretic peak resolution is optimized.

There are several injection methods that can be used in MCE. Commonly used by researchers are the dynamic, gated, floating, and pinched electrokinetic injection methods—each with their respective advantages and disadvantages [61], [62]. Dynamic and gated injections use a continuous flow of the sample solution allowing for real-time changes to the flowing sample volume by controlling the applied voltage and actuation time. A disadvantage of continuous flow-based injection methods is the large injection bias (i.e., the varying electrophoretic mobility of the analytes) and poorly defined sample volume in contrast to pinched injection methods. Injection bias and sample volume variability impede the reliability of quantitative measurements in MCE. For the case of bias injection, literature has shown that UV fluorescence-based detection renders injection bias negligible [63]. Both pinched and floating injection methods ensure accurate and non-varying sample volume injection—the latter allowing slightly more sample volume. Additionally, a pinched sample yields high separation efficiency of the analytes within the sample solution which can be leveraged to detect multiple analytes within a sample. Literature has shown the feasibility in detecting multiple analytes—such as levofloxacin and norfloxacin—from a single sample [64], [65], [66]. High separation efficiency of analytes with low dispersion are ideal conditions for fluorescence detection [61], [62]. An MCE

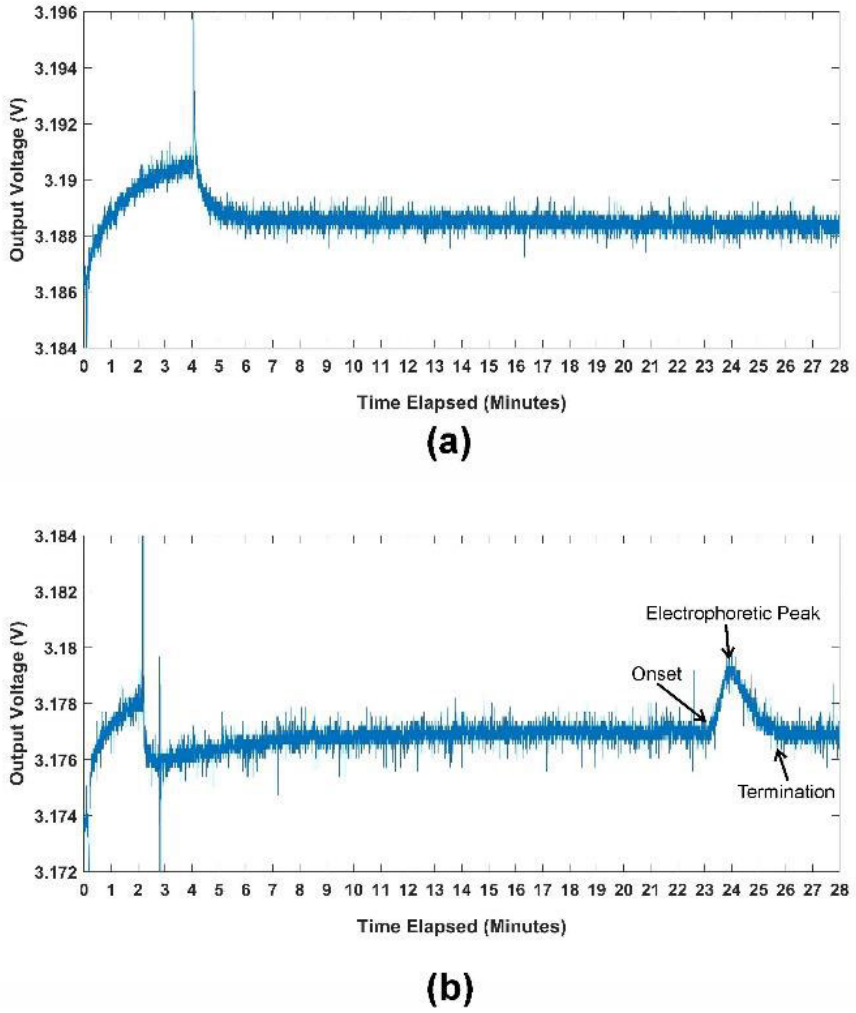

FIGURE 6. Time-domain data of MCE experiments are shown, where (a) a sample of milk without ciprofloxacin is tested, and no electrophoretic peak is displayed, followed by (b) a sample of milk injected 0.5 mM of ciprofloxacin, where a clear bell-shaped electrophoretic peak is seen. This electrophoretic peak is fluorescence detection of ciprofloxacin in the milk sample.

system with increased channel width [67] shows potential use for unfiltered milk, however, this is not explored in this work.

In our previous work, we developed a MCE antibiotic detection system that was tested for multiple concentrations of ciprofloxacin in milk [11]. This experiment is performed again here. A trial of 0.5 mM of ciprofloxacin using the previous (no lock-in amplifier) unoptimized MCE system is shown in Fig. 6. The presented results use a similar system to that of Bosma et al. [11] and the recorded data differs slightly. The rapid change in electric field involves an equivalent, measurable voltage spike. The measured voltage change corresponds to the lab-on-a-chip system transitioning between the pinched sample injection condition and electrophoretic condition. The corresponding voltage spike that is induced to change between system conditions are depicted at 4 minutes in Fig. 6(a) and 2 minutes in Fig. 6(b).

A blank milk sample (i.e., no antibiotics) is depicted in Fig. 6(a) where no notable electrophoretic peaks are detected. In contrast, the trial with 0.5 mM of ciprofloxacin in the milk shows a clear bell-shaped electrophoretic peak occurring at approximately 24 minutes, as shown in Fig. 6(b). This peak represents the point where only the ciprofloxacin within the pinched sliver of milk sample passes the detection region. This MCE technique for dairy applications has been previously demonstrated in Bosma et al. [11], and the reintroduced MCE and FS system here is a successful and further demonstration that this lab-on-a-chip antibiotic detection system is feasible. Directly comparing the signal without lock-in amplification in Fig. 2 with that of the unoptimized MCE system is not intended for this discussion as the latter was performed with a different concentration (0.5 mM), optical power of 2.4 mW for the UV LED, and photodiode.

The results of Bosma et al. [11] are to be extrapolated using our comparison of with and without LIA integration in off-chip measurements. Using the improvement ratio previously established, $LOD_{DC}/LOD_{LIA} = 2200$, it is predicted that integration of our LIA sensor with MCE system of Bosma et al. could lower LIA and LOQ to values of 2.28×10^{-2} mg/kg and 9.31×10^{-2} mg/kg, respectively. This estimated performance compares very favourably with the regulatory limit and other LOD and LOQ values from Table 1. Such an advancement has great potential to enhance modern bio-microsystem sensors [68], [69]. Our work also has the potential to advance beyond lock-in fluorescence systems that were not largely applied for biological applications [70].

VIII. ANTI-INTERFERENCE

The primary measurement in the presented sensor is confirmed by comparing the measured signal with a baseline sample. In this sense, a recording was performed without any antibiotics, to confirm that there are no constituents which can be detected by the FS detection system. Thus, anti-interfering is confirmed. For the envisioned application, a farmer should have knowledge of the antibiotic to treat the cattle. Therefore, only one antibiotic is analysed. In cases where a cocktail of antibiotics is prescribed, some separation of analytes would be required, such as is achievable in MCE devices. Integration MCE an important point of future work.

With respect to anti-interference with other antibiotics, this can be implemented in future studies, with integration with MCE. Multiple antibiotics can be detected with a single excitation source [71]. Multiple antibiotics can be differentiated based on the times which the electrophoretic peaks pass by the detection region, indicating separation time. Separation time would be directly proportional to the charge-to-mass ratios, which is dependent on the chemical structure of the antibiotic. An antibiotic with a charge-to-mass ratio for quick separation, would be observed earlier than others [14], [15]. This would require establishment of separation times within a given sensor, dependent on applied electric fields and design of the microfluidic chip. The concentration of the detected antibiotic would be proportional to the detected amplitude, but the separation time would remain consistent, based on the antibiotic's chemical structure.

Another method for anti-interference would be to implement multiple detection sources within the same system. These detection sources would be specific to the fluorescence emission of the given analytes, so the detected concentrations of multiple antibiotics can be distinguished based on the electrophoretic peaks of the various detection sources.

IX. CONCLUSION

This work introduced a sensor for dairy antibiotic detection of ciprofloxacin in milk. Our sensor integrated florescence spectroscopy and lock-in amplification and was found to be capable of detection below the regulatory limit. We compared the system to one without the integrated lock-in amplification, as in traditional fluorescence sensors. We provided MCE results and placed our work in this context, for potential future integration with MCE. The future impact of this antibiotic detection system will allow a fast and quantifiable detection method for antibiotics in milk. The ability to obtain rapid analysis times, miniaturize the sensor, and full automation of the system would reduce dairy waste for farmers. Current manual methods require testing on a full milk repository, as it is unfeasible to test on individual cows. The implementation of rapid and automated sensing would allow farmers to quickly test milk samples from each cow, thus allowing the farmer to exclude a single cow's milk from the full milk repository if it is contaminated. Given the development of recent miniaturized LIA systems, its deployment on farms is a feasible option. This study provided an important dairy sensor, with potential to significantly improve dairy sensing of antibiotics.

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