Alternating Current Voltammetry at a Bipolar Electrode with Smartphone Luminescence Imaging for Point-of-Need Sensing

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Keywords
bipolar electrochemistry, alternating current voltammetry, electrochemiluminescence, smartphone detection

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Alternating Current Voltammetry at a Bipolar Electrode with Smartphone Luminescence Imaging for Point-of-Need Sensing

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Abstract

Bipolar electrochemistry allows for facile arraying of tens to thousands of electrochemical sensors that can be controlled by a single pair of driving electrodes. While bipolar electrodes (BPEs) have been applied to many sensing motifs, their sensitivity and specificity are limited by the lack of diversity in voltammetric methods that have been developed for these wireless electrodes. In this study, electrochemiluminescence (ECL) from the co-oxidation of Ru(bpy)$_3^{2+}$ and tripropylamine (TPA) is evaluated as a reporting reaction for alternating current voltammetry (ACV) on a BPE at frequencies of 1.0 Hz and 5.0 Hz. We observe sinusoidal alternating luminescence that follows a similar trend to that of the simultaneously monitored current – a plot of the amplitude versus potential approximates a bell-shaped curve. Notably, the luminescent response to the current is detected with a smartphone, which underscores the portability of this method. The fidelity of the transduced signal is determined both in a traditional 3-electrode configuration and at a BPE. These experimental results indicate that the alternating luminescence follows the current sufficiently for quantitative sensing but is diminished at higher frequencies and peaks at a shifted potential. These results are significant because they demonstrate the potential for application of ACV at BPE arrays for multiplexed point-of-need sensors and provide guidance for the selection of reporting reactions in this context.
1. Introduction

In this paper, we evaluate electrochemiluminescence (ECL) as a reporter for signal obtained during alternating current voltammetry (ACV) at bipolar electrodes (BPEs). We demonstrate that an AC voltammogram can be constructed from the alternating luminescent signal and that the peak amplitude is linearly correlated to the analyte concentration detected under mass transfer limited conditions at the opposing end of the BPE. The underlying mechanisms for deviation of the luminescence from the current are discussed. These results are significant because the adaptation of AC voltammetric methods to BPEs will expand their potential application in multiplexed point-of-need (PON) sensing. Finally, to underscore the suitability of this method for PON applications, we utilize a smartphone camera to monitor the signal, and find that critical features of the luminescence ACV are captured with sufficient accuracy for analysis.

BPEs lack direct contact to an external power supply and are readily arrayed. Therefore, many BPEs can be actuated simultaneously with a single pair of driving electrodes. In this context, the current is reported by a faradaic reaction that generates a visible signal such as ECL.[1] These features make BPEs especially well-suited to multiplexed sensing in PON applications, in which clinical or environmental analytes are quantified on site, thereby allowing for immediate intervention.[2] A growing body of work is dedicated to adapting smartphones as PON tools, because of their low cost, portability, and accessibility.[3–9] Detection of colorimetric,[5] fluorescent,[5] electrochromic,[4] and luminescent[6–9] signals with a smartphone have been demonstrated, and there are several examples of smartphone detection of reporting reactions on BPEs in the literature.[7,9] However, some of the most sensitive and information-rich voltammetric methods employ an alternating sinusoidal or square-wave potential, which are currently not developed for BPEs. Several recent examples illustrate the enhanced signal
afforded by ACV in the context of electrochemical sensing of clinical and environmental analytes.\textsuperscript{[10–17]} Such sensors meet many of the requirements for PON devices, in that they are reagent-less, reusable, relatively fast, and sensitive.\textsuperscript{[11]} Therefore, ACV, in the context of BPEs, creates a promising platform for multiplexed PON sensors.

In recent years, there has been increased adaptation of voltammetric techniques to BPEs.\textsuperscript{[18–23]} Of these, few have explored alternating current. Of note is the Shannon group’s use of a square wave potential to drive ferricyanide reduction, and separately for DNA sensing, at the cathode of a Au BPE with an ECL readout at the anode, using Ru(bpy)\textsubscript{3}\textsuperscript{2+} and sodium oxalate in aqueous solution.\textsuperscript{[18]} The ECL response to a 0.5 Hz square wave signal was recorded and averaged over several oscillations to improve the signal-to-noise ratio.\textsuperscript{[18]} A LOD of 300 fmol/cm\textsuperscript{2} was determined for methylene-blue-conjugated DNA in a self-assembled monolayer on the BPE.\textsuperscript{[18]} Although square wave voltammetry (SWV), which has a gradually varied DC potential, was reported for a 3-electrode configuration, no such DC bias was applied in the BPE configuration. Eßman and coworkers studied the application of an AC technique to a BPE in a different way; they rotated a graphite rod BPE in an aqueous solution of luminol under a constant electric field applied by stainless steel driving electrodes.\textsuperscript{[21]} The rotation of the long axis of the rod relative to the electric field induced an AC response. This alignment occurred twice per rotation, and therefore, at 100 rpm, the effective frequency was \(2f = 3.2\) Hz. Rotation was adopted as an alternative to an AC driving voltage to circumvent the large time constant of the driving electrode solution interface.\textsuperscript{[21]} Similarly, Sojic and coworkers developed a rotating BPE with ECL readout. In this case, the rotation was induced on a gold-coated iron wire with a magnetic field rather than with a mechanical rotor. A higher rotation rate (255 rpm) was employed, with effective frequency of 8.5 Hz. Although this open BPE configuration results in a
spatially varied interfacial potential along the length of the BPE at any instant, the resulting signal is unlike ACV in two important ways. First, at this rotating BPE there is no DC component (DC offset) to the applied potential, as in ACV. Instead, at all points along the BPE the interfacial potential is oscillating about zero. Second, the AC component varies in amplitude along the BPE, while in ACV the amplitude is constant.[23] Despite these advancements, AC voltammetric techniques on BPEs, and their fidelity to analogous techniques carried out in a 3-electrode cell, have yet to be demonstrated.

There is also a body of existing work that evaluates the response of non-aqueous ECL reactions to an AC potential applied between two closely spaced electrodes.[24–26] This research examines mechanisms of ECL in non-aqueous solvents, in which light is emitted following interaction between the oxidized and reduced states of a single species.[24] The results of these studies indicate that the ECL response to an AC potential is faster,[24] more efficient,[24] and more sustained[25] than that obtained with a DC potential. However, the response of aqueous ECL of Ru(bpy)$_3^{2+}$ with co-reactant tripropylamine (TPA) to a sinusoidal AC potential has not been evaluated for similar features.

In the present study, we evaluate the fidelity of the light intensity emitted by ECL, arising from the co-oxidation of Ru(bpy)$_3^{2+}$ and TPA in an aqueous environment, to the magnitude of faradaic current obtained during ACV at a pyrolyzed photoresist film (PPF) electrode.[27] In contrast to previous work, we report on an AC voltammetric technique, not an amperometric technique, and at a frequency of 5.0 Hz, a higher frequency than previously demonstrated on BPEs. To avoid the large time constants at driving electrodes associated with the oscillating potential, Ag/AgCl (non-polarizable electrodes) are employed as driving electrodes. Throughout this study, a smartphone with inexpensive adaptations is used to collect the fluctuating light from
the ECL reaction with sensitivity sufficient for accurate data analysis. We first compare the luminescence to the current arising from ACV of the ECL reaction in a 3-electrode cell configuration. Under these conditions, the amplitude of the alternating luminescence peaks 50-100 mV earlier than does the amplitude of the current. The identification of this shift is critical to the correct interpretation of results obtained by this method when subsequently applied to bipolar electrochemistry. Second, we employed ECL as a reporting reaction for ACV of a coupled reaction on a BPE (Scheme 1). Our results indicate that the luminescence-to-current ratio (efficiency) is more than 28% greater for the AC component of the current relative to that obtained by a DC technique and that the top of the linear range of ferricyanide detection is dictated by the ability of the ECL reaction to oscillate. We conclude that ECL reactions that are brighter and more kinetically facile are needed to extend the linear range of ACV at BPEs.

2. Theoretical Background

Bipolar electrochemistry. A BPE is a conductive substrate that lacks ohmic contact to a power supply (is wireless) and is immersed in an electrolyte solution.[28] If a sufficiently high electric field is applied to the solution, opposing ends of the BPE facilitate electrically coupled faradaic reactions, which occur simultaneously and with equal magnitude current. When the two ends of each BPE are fluidically separated by an insulator as is depicted in Scheme 1b (cross-sectional side view), equation (1) governs the minimum applied potential bias ($\Delta E_{onset}$) required for onset of the paired redox reactions:

$$\Delta E_{onset} = (E_{drive,a}^{0r} - E_{drive,c}^{0r}) + (E_{BPE,a}^{0r} - E_{BPE,c}^{0r}) + i_{BPE}R_s$$  (1)
Where $E^\theta_{\text{drive,}a}$, $E^\theta_{\text{drive,}c}$, $E^\theta_{\text{BPE,}a}$, and $E^\theta_{\text{BPE,}c}$ are the formal potentials of reactions occurring at the driving and BPE anode $(a)$ and cathode $(c)$.\[^{[29]}\] The term $i_{\text{BPE}}R_s$ represents the ohmic potential drop across the solution resistance ($R_s$) along the path of the current to the BPE ($i_{\text{BPE}}$). This quantity is highly dependent on the design of the cell and the conductivity of the electrolyte\[^{[19]}\] and was determined to be negligible for all experiments reported here. A single pair of driving electrodes can supply the potential for an array of tens to thousands of BPEs, operated in parallel.\[^{[30]}\] It has been experimentally demonstrated for both an open\[^{[31]}\] and closed\[^{[32]}\] BPE array that the individual electrodes behave essentially identically. This feature of BPEs makes them advantageous for multiplexed sensing.

**Electrochemiluminescence.** Because BPEs are wireless, a current readout for each BPE cannot easily be obtained. To mitigate this issue, a sensing reaction occurring at one end of the BPE is coupled to a reporting reaction, which generates a visible signal, at the other end. In such case, a closed BPE configuration is frequently employed to eliminate interference or mixing between redox species.\[^{[33]}\] Several reporting reactions have been devised to transduce an electronic current into a visible signal.\[^{[34,35]}\] One of the most prominent reporting reactions is the ECL reaction between Ru(bpy)$_3^{2+}$ and TPA, shown in equations 2-5.\[^{[27,29,36]}\]

\[
\begin{align*}
Ru(bpy)_3^{2+} & \rightarrow Ru(bpy)_3^{3+} + e^- \quad (2) \\
TPA & \rightarrow [TPA^-]^+ + e^- \rightarrow TPA^- + H^+ \quad (3) \\
Ru(bpy)_3^{3+} + TPA^- & \rightarrow Ru(bpy)_3^{2+} + products \quad (4) \\
Ru(bpy)_3^{2+} & \rightarrow Ru(bpy)_3^{2+} + hv \quad (5)
\end{align*}
\]
One reason that this reaction is so widely used for reporting at BPEs is that the intensity of the luminescence from this reaction is directly proportional to faradaic current, as determined by chronoamperometry (CA). When employed in a PON device, this feature of ECL simplifies data interpretation.

*Alternating current voltammetry.* Here, we investigate ECL in the context of ACV. In this technique, a DC potential ($E_{DC}$) is stepped, setting surface concentrations of redox species. An additional AC potential ($E_{AC}$) is applied at an angular frequency ($\omega$), which is $2\pi$ times the conventional frequency in Hz ($f$), resulting in perturbations of these concentrations. It is a highly precise electroanalytical method, which inherently subtracts a portion of the capacitive current because only the amplitude ($I$) of the AC current ($i_{AC}$) is analyzed (although square wave voltammetry was specifically adapted from AC polarography to completely background subtract capacitive current). The peak amplitude of the alternating current ($I_p$), located at a potential defined as $E_p$, in a reversible reaction is proportional to several quantities, outlined in the following equation.

\[
I_p = \frac{n^2F^2A\omega^{1/2}D_{O}^{1/2}C_O^*\Delta E}{4RT}
\]  

(6)

Where $n$ is the number of electrons, $F$ is the Faraday constant, $A$ is the area of the electrode, $D_O$ is the diffusion coefficient of the oxidized species, $C_O^*$ is the bulk concentration of the oxidized species, $\Delta E$ is the amplitude of $E_{AC}$, $R$ is the ideal gas constant, and $T$ is the temperature. Note
that for the reversible case, $I_p$ is proportional to $\omega^{1/2}$, whereas for a slow reaction (or high frequency), irreversible behavior leads to frequency independence.

3. Results and Discussion

Evaluation of ECL intensity following a DC potential step. Prior to evaluation of the ECL response to an AC perturbation, we first examine the correlation between the luminescence intensity and current obtained at the PPF microelectrode, in a 3-electrode cell configuration, under DC conditions. Figure S1 (Supporting Information, SI) is a plot of the time-averaged luminescence and current densities resulting from the ECL reaction (5.0 mM Ru(bpy)$_3^{2+}$, 25.0 mM TPA, 0.5 M phosphate buffer, pH 6.9) following a DC potential step. The range of potentials applied to obtain these responses was from 1.0 V to 1.5 V vs. Ag/AgCl, and the light and current data were averaged over the period $t = 1.0$ s to 1.5 s after the potential was applied. The data presented are from two sets of experiments, each performed in triplicate, using separately a microscope (squares) at 20 frames per second (fps) and a smartphone (triangles) at 24 fps as the detector for the luminescence. The results show a linear relationship between ECL intensity ($I$) and current density ($j$) in a range from 0.7 (±0.2) to 2.9 (±0.3) mA/cm$^2$ for the microscope and 0.3 (±0.1) to 1.4 (±0.2) mA/cm$^2$ for the smartphone. Crooks and coworkers also reported a linear relationship between ECL intensity and current for this reaction at a Au BPE over the range of current densities (about 0.03 – 0.06 mA/cm$^2$) with a more sensitive camera and at longer exposure times.$^{[1]}$ These results are significant because they identify a range over which ECL intensity is linearly related to direct current, and further, the slope can be used to evaluate the efficiency with which current is converted to counts for each detector. Within the linear range
of ECL response obtained during CA, for the microscope, the luminescence-to-current ratio is 7.7 x 10⁸ counts/mA, and for the smartphone, 3.5 x 10⁸ counts/mA.

*Evaluation of ECL intensity during ACV in a 3-electrode cell.* We next evaluated the accuracy with which ECL transduces the current of an AC voltammogram to light by simultaneously monitoring the current and the luminescence obtained during ACV of this ECL reaction at a PPF electrode in a 3-electrode configuration. At $t = 0$ s, the potential program was initiated, such that first, $E_{DC}$ was stepped to 1.0 V and maintained for $1/2f$ after which an AC perturbation ($\Delta E = 50$ mV, $f = 1.0$ or 5.0 Hz) was applied. Successive steps in $E_{DC}$ had a magnitude of 0.01 V and occurred at an interval of 2.0 s. The final potential step was $E_{DC} = 1.5$ V. Videos showing the oscillation of the luminescence are available as supplementary files **Movies M1** (1.0 Hz) and **M2** (5.0 Hz). The luminescence intensity is nearly uniform across the surface of the electrode and flashes in response to the alternating potential.

**Figure 1a,c** are plots of the ECL intensity versus time obtained by a smartphone camera (24 fps) during ACVs obtained at 1.0 Hz and 5.0 Hz, respectively. The plotted intensities have been averaged across the exposed area of the PPF electrode. At low overpotentials, the potential is just sufficient to drive the light-emitting reaction, and therefore the amplitude of the transduced signal is small for the forward perturbation (crest) and no luminescence is observed for the backward perturbation (trough). As $E_{DC}$ is increased, a full sine wave oscillation develops. The amplitude of the luminescence continues to increase and then peaks. At high overpotentials, no oscillations in luminescence are observed, while a sinusoidal current remains. Finally, the steady (‘DC’) luminescence peaks at $E_{DC} = 1.24$ V for both the 1.0 Hz and 5.0 Hz trials and then undergoes a decay, which we attribute to self-quenching.⁴¹
Figure 1b,d show the amplitude of the alternating current density ($I$) and luminescence density ($L$) plotted against $E_{pc}$ for 1.0 Hz and 5.0 Hz perturbations ($n = 3$, separate devices). This representation makes it apparent that $E_p$ is shifted by about 90-100 mV earlier for the luminescence than for the current. We hypothesize that at these very positive overpotentials, the generation of TPA$^\cdot$ is mass transfer limited and insensitive to AC perturbations, and instead the oscillations in current arise from modulation of the rate at which Ru(bpy)$_3^{3+}$ is produced. A lack of oscillation in luminescence signifies that it is TPA$^\cdot$ and not Ru(bpy)$_3^{3+}$ that is the limiting intermediate in photon production at these potentials. This interpretation is supported by ACV of separate solutions of each individual reagent, which shows that $E_p$ occurs about 20 mV earlier (less positive) for TPA than for Ru(bpy)$_3^{2+}$. These results imply that an increased concentration of TPA would lessen the shift in $E_p$ between luminescence and current. However, such an increase is impractical due to its limited solubility.

According to eq. 6, as frequency increases, $I_p$ (and $J_p$) should also increase. Figure 1c,d show that an increase in frequency from 1.0 Hz to 5.0 Hz leads to an increase in $J_p$ from 0.27 mA/cm$^2$ to 0.32 mA/cm$^2$ (half of the expected increase for a reversible reaction). However, the same trend is not observed in $L_p$ for the two frequencies. As the frequency increases, a decrease in $L_p$ is observed. This decrease in $L_p$ with increased frequency may be caused by a delay in the onset of the light emitting reaction. However, further experiments are needed to explain this phenomenon. The data of Figure 1 was obtained at 24 fps, which is a standard frame rate available on most smartphones (while some models image at up to 60 fps). This sampling rate is sufficient for 1.0 Hz but under-samples the 5.0 Hz signal (4 data points per wave), leading to signal attenuation. Therefore, current smartphone frame rates render frequencies > 5.0 Hz inaccessible for PON applications. Most importantly, it can be concluded from the comparison of
data obtained at several frame rates (Figure S2) that the decrease in $L_p$ from 1.0 Hz to 5.0 Hz occurs regardless of frame rate.

Finally, by dividing $L_p$ by $J$ (at the same potential), we find that the luminescence-to-current ratio for the AC component is $4.5 \times 10^8$ counts/mA and $3.7 \times 10^8$ counts/mA at 1.0 Hz and 5.0 Hz, which are 28% and 5% higher than in CA, respectively. This result implies an increase in the efficiency with which the signal is transduced under an alternating voltage. Both frequencies are similarly increased when the signal attenuation caused by under-sampling is taken into consideration.

Based on comparison of these results with those obtained by light microscopy under matched conditions (Figure S3), we conclude that although the smartphone is not as sensitive as the microscope, it is still sufficiently sensitive to detect the light oscillations needed to reproduce the key features of an AC voltammogram. Therefore, this ECL reaction can be used to report the current of ACV with smartphone detection.

Fundamental aspects of ACV in the context of bipolar electrochemistry. In bipolar electrochemistry, two faradaic reactions are electrically coupled by the BPE, and therefore, we consider the potential bias applied by the driving electrodes ($\Delta E_{tot}$) instead of absolute potentials. Scheme 2a shows linear sweep voltammograms for the two faradaic reactions considered in the present study – the oxidation of the Ru(bpy)$_2^{2+}$/TPA system (right side) and the reduction of ferricyanide (left side). The data shown here was obtained at a PPF microelectrode in a 3-electrode cell at a scan rate of 50 mV/s versus a Ag/AgCl/sat’d KCl reference and with a Pt wire auxiliary electrode. These two reactions are plotted together here to illustrate that when two such faradaic processes are coupled and occur at opposing ends of a BPE, the rate of each
reaction must satisfy the requirement that \( i_{BPE} = i_c = -i_a \). When this condition is not met, excess charge accumulates on the BPE, thereby shifting \( E_{BPE} \) until \( \Delta E_{BPE} \) spans potentials along these \( i-E \) curves at which the cathodic and anodic currents are equal in magnitude (see matching pairs of markers, Scheme 2a).

Scheme 2b shows raw (unprocessed) AC voltammograms obtained for the same two faradaic reactions. The key feature of these voltammograms is that the maximum amplitude oscillation in the current occurs at about 1.2 V and 0.33 V vs. Ag/AgCl for ECL and ferricyanide, respectively. These maxima, \( J_p \), occur where the reactions are the most symmetrically responsive to the AC perturbation, which for a reversible reaction is at the half-wave potential, \( E_{1/2} \). When these two reactions are coupled via a BPE, there are three important features of the process to consider. (1) The magnitude of the DC component of the current must be matched \( (i_c = -i_a) \), but at the value of \( E_{BPE} \) that satisfies this requirement, the AC amplitude for the two processes may not be equal. In this scenario, we hypothesize that the smaller of the two AC currents is limiting. However, other factors, such as oscillation of \( E_{BPE} \) or a mismatch in phase between the AC currents may influence the amplitude of the coupled processes. (2) Because \( E_{BPE} \) is able to float, the potential axis of the ACV, which displays \( \Delta E_{tot} \), may not be linear with respect to absolute potentials (versus a reference). The half-wave potential of the reporting reaction serves as an internal reference, and therefore, a reporting reaction with a very steep onset and a high limiting current will yield a voltammogram nearest the ‘true’ curve that would be obtained in an ideal 3-electrode cell. (3) While the DC component of the reporting reaction (ECL) may be sufficiently large to allow the sensed reaction (ferricyanide) to limit the overall current, the AC component may be insufficient. In the experiments conducted at a BPE
and reported in subsequent subsections, we find that the inability of the ECL reaction to oscillate limits the linear range of this technique.

Brackets on the curve obtained for ferricyanide (left side, Scheme 2b) indicate the amplitude obtained for ferricyanide at three distinct values of $E_{DC}$. These amplitudes must be faithfully represented by the ECL intensity to generate a ‘luminescence AC voltammogram’ for ferricyanide. Scheme 2c is a plot of the ECL intensity resulting at the anodic pole when the two faradaic reactions in Scheme 2b are coupled via a BPE. The brackets indicate $L$ that when plotted versus $E_{DC}$ yields the AC voltammogram shown in Scheme 2d. This AC voltammogram indicates that an $L_p$ of $3.3 \times 10^7$ counts/cm$^2$ occurs at $\Delta E_{tot} = 0.87$ V, which in this case is equal to the distance between $E_p$ for the individual reactions ($1.2$ V – $0.33$ V = $0.87$ V). These results are significant because they show that ECL reproduces an ACV on a BPE in that the luminescence rises and falls in amplitude as a function of the AC potential. In the following subsections, we first compare the sensitivity of ACV with a DC technique when carried out at a BPE. Then, we examine the influence of experimental factors on the shape and peak position ($\Delta E_{p,L}$) of this luminescence ACV obtained at a BPE, and identify the range over which $L_p$ is linearly correlated to ferricyanide concentration (eq. 6).

ECL response to chronoamperometry on a BPE. CA was chosen for comparison with ACV carried out at a BPE. In both cases, the closed BPE configuration illustrated in Scheme 1 was employed, and as shown, the cathodic and anodic reservoirs were filled with ferricyanide solution and the ECL mixture, respectively. Ag/AgCl/sat’d KCl driving electrodes were dipped into each of the two reservoirs. An ammeter connected the two poles of the BPE, which extended into two fluid filled compartments. To obtain this configuration, a PDMS monolith with
imprinted channels was aligned on top of the BPE poles, such that a 1.0 mm² electrode area was exposed to the fluid in the channels on each end. The smartphone camera with lens adaptation was affixed above the microfluidic device, and the camera was focused on the BPE anode (for smartphone images of the device, see Scheme 3a and 3b).

To obtain a chronoamperogram, $\Delta E_{tot} = 1.2$ V was applied at the driving electrodes, as this potential bias exceeds the $E_p$ values for the coupled reactions, and reaches the mass transfer limited regime. This potential program was applied sequentially to each of ten distinct concentrations of ferricyanide (0.5 to 15.0 mM in 2.0 M KCl) present as a quiescent solution in contact with the sensing (cathodic) end of the BPE. The resulting current and luminescence responses were averaged for 0.5 s, taken 1.0 s after the potential was applied.

The time averaged response in $j$ and $l$ that resulted from each of the ferricyanide concentrations is plotted in Figure 2. At low concentrations of ferricyanide, a linear response is observed from both the current and luminescence. This trend occurs because the concentration of the ferricyanide is limiting the current for the BPE. At high concentrations, the current becomes limited by the ECL reaction and is insensitive to ferricyanide concentration. Based on these results, we conclude that the linear dynamic range for luminescence response on the BPE with CA is 0.5 mM to 5.0 mM with an LOD of 0.61 mM. The lower limit is dictated by the relatively low sensitivity of the smartphone camera.

_ECL as a reporting reaction for ACV of a second reaction coupled via a BPE._ We next evaluated the ability of the ECL reaction to report the current obtained during ACV at a BPE. A critical question is whether the luminescence faithfully reproduces the current, and therefore, we again employed a split BPE configuration so that the current and luminescence could be monitored.
simultaneously (Scheme 1). Ten distinct ferricyanide solutions were analyzed in the sensing channel, while the ECL reaction mixture filled the reporting channel.

Scheme 3d is a smartphone image of the luminescence obtained at 1.0 Hz. Videos of the oscillating luminescence are included in the SI as Movies M3 (1.0 Hz) and M4 (5.0 Hz). It is notable that the luminescence intensity is less uniformly distributed across the electrode surface in this BPE configuration than in the 3-electrode cell (Scheme 3c), which we interpret as arising from differences in current distribution. Figure 3a,b show $j$ and $l$ obtained at the BPE anode for the detection of 3.0 mM ferricyanide at the BPE cathode with 1.0 Hz frequency. The signals observed with this BPE configuration were similar to those obtained with the 3-electrode cell in that $J_p$ and $L_p$ rise and fall, here with increasing $\Delta E_{tot}$, allowing an AC voltammogram to be obtained. However, a notable difference is that the relative magnitude of the AC component of the luminescence is lower with respect to the ‘DC’ component (steady luminescence), when comparing this BPE data to that obtained in a 3-electrode cell (compare Figure 3b and Figure 1a).

Figure 3c shows a representative overlay of $L$ and $J$ for an AC voltammogram of a 3.0 mM ferricyanide solution in the BPE configuration. Interestingly, on a BPE, $\Delta E_{p,L}$ is greater (requires a larger overpotential) than the potential bias at which the amplitude in the current density peaks ($\Delta E_{p,J}$). This trend is the opposite of that observed for the ECL reaction when interrogated alone in the 3-electrode cell.

When using a BPE configuration, the reporting reaction must be limited by the sensing reaction. Therefore, as long as the amplitude of the current from the sensing reaction ($J_s$) is sufficiently high to result in light emission and does not exceed that of the reporting reaction ($J_r$), we anticipate that the ECL reaction signal will yield quantitative information about the sensing reaction. To characterize the linear range of this technique, we plotted $J_p$ and $L_p$ obtained for
each of the ten concentrations of ferricyanide sensed on the BPE cathode in response to an ACV at 1.0 Hz (Figure 4a) or 5.0 Hz (Figure 4b). The linear dynamic range was found to be about 0.5 mM to 4.0 mM for both \( J_p \) and \( L_p \) at both frequencies. There is a linear relationship between \( J_p \) and \( L_p \) with low concentrations of ferricyanide. This trend appears because the reduction of ferricyanide is limiting the amount of current that flows through the BPE. At high concentrations of ferricyanide, \( J_p \) and \( L_p \) become limited by the reporting reaction, despite the fact that the effective concentration of the ECL solution is higher than that of the ferricyanide solution.

To understand the limiting behavior, we separately considered the ability of ECL to provide the DC and AC components of the current. These components (\( J_{DC} \), dashed lines and \( J \), solid lines) are plotted for the reduction of 7.0 mM ferricyanide (Figure S4a) and for the ECL reaction (Figure S4b) in a 3-electrode cell. While the peak DC current is higher for ECL than for ferricyanide, the \( J_p \) is lower due to insufficient reversibility of the ECL reaction. In other words, the ECL reaction is kinetically slower than ferricyanide reduction. An important conclusion that can be drawn from these results is that the ability of the Ru(bpy)_3^{2+}/TPA system to report ACV at BPEs is kinetically limited, at the upper end. Therefore, reporting reactions that permit a higher concentration of reagents (especially the co-reactant) or is more reversible, would be more compatible for this system. Finally, it should be noted that a second peak in \( J \) appears at high concentrations of ferricyanide (Figure S5a). We believe that this peak is caused by the ability of the BPE to shift potentials to a different paired reaction (water oxidation) to compensate for the increased current from the high concentration of ferricyanide.

We also compared the luminescence obtained in the two electrode configurations. At the BPE, \( L_p \) approached a limit at 3.6 x 10^7 counts/cm^2 (Figure 4a) which is approximately 36% of the maximum in \( L_p \) observed for ECL alone in a 3-electrode configuration (Figure 1c). Therefore,
it is clear that the ECL reaction is capable of generating a greater amplitude luminescence than what we observe at the BPE. However, the ratio of $L_p$ to $J_p$ (photons per electron) is about 2.6X lower on a BPE than for ECL in a 3-electrode cell. This loss in $L_p$ must be caused by features unique to a BPE configuration, and therefore, may result from a non-uniform distribution of current density at the BPE anode (leading to a fraction of the area below the threshold current density for ECL), a mismatch in phase between the two coupled reactions, or from more extensive charging at the additional surface (the BPE cathode), which in 2.0 M KCl is expected to have a large double layer capacitance. This effect is the focus of ongoing studies in our laboratory.

4. Conclusions

In this study, we demonstrated the response that the ECL reaction between Ru(bpy)$_3^{2+}$ and co-reactant TPA has to ACV performed in both a 3-electrode and BPE configuration. The luminescent AC voltammogram was detected with a smartphone camera, and in the 3-electrode cell, verified with light microscopy. It was determined that the smartphone is sufficiently sensitive to detect a luminescent AC signal and to accurately reproduce the features of an AC voltammogram. Upon performance of an ACV at 1.0 Hz in the 3-electrode configuration, the efficiency of the transduced signal was improved by 28% relative to that obtained with CA. We further determined that the luminescence response from the ECL reaction when paired on a BPE with a facile reaction (ferricyanide) provided a linear response in the peak amplitude of the luminescence ($L_p$) over a range of 0.5 mM to 4.0 mM ferricyanide. By comparing the AC and DC components of each of the paired reactions, we concluded that the upper concentration limit is caused by slow kinetics of the ECL reaction. Despite an increase in efficiency with which the
current is transduced to luminescence with the AC technique, the LODs for ACV and CA were similar. In the future, the limitations discussed here will be addressed by optimizing the ECL reaction for AC conditions to expand the utility of ACV in BPE-based platforms. Our results indicate that with further optimization, the performance of ACV on arrayed BPEs with smartphone imaging will serve as a viable option for multiplexed PON sensors.

5. Experimental Section

Chemicals. The following chemicals were purchased and used as received: potassium ferricyanide (>99% (Acros Organics, Morris, New Jersey), tris(2,2-bipyridine)ruthenium (II) >98% (Ru(bpy)_3^{2+}) (Strem Chemicals, Newburyport, MA), tripropylamine >98% (Tokyo Chemical Industry, Tokyo, Japan). The ECL reaction solution was prepared with 5.0 mM Ru(bpy)_3^{2+} and 25.0 mM TPA in 0.5 M Phosphate buffer. The ferricyanide solutions were prepared with 2.0 M KCl. All electrolyte salts were purchased from Fisher Scientific (Waltham, MA) and diluted with double deionized water (18.2 MΩ cm, Sartorius Arium Pro, Göttingen, Germany).

Pyrolyzed photoresist film (PPF) electrode preparation. PPF electrodes were fabricated on quartz slides following a procedure outlined by Brazill and coworkers.[42] Briefly, positive photoresist (AZP4620, MicroChemicals GmbH, Ulm, Germany) was spin coated for 30 s at 3000 rpm on quartz slides that had been cleaned in a base bath. Following spin coating, the slides were soft baked at 110 °C for 1 min, then exposed to UV radiation at 350 mJ/cm². The slides were then developed in 4:1 (v:v) AZ 400K developer (EMD, Somerville, New Jersey). Slides were
individually pyrolyzed in a tube furnace (Lindberg Blue M, Thermo Scientific) with a quartz tube flushed with 95% \( \text{N}_2 \) and 5% \( \text{H}_2 \) (Airgas, Radnor, PA) forming gas. The tube was flushed for 20 min at RT at 50 cm\(^3\)/min, then the temperature was ramped at 10 °C/min to 1000 °C. 1000 °C was held for 1 h, then left to cool to room temperature with forming gas flowing.

Microfluidic device preparation and operation. Standard soft lithography practices were used to fabricate microfluidic channel molds.\[^{[43]}\] Negative photoresist (SU-8 2050, Microchem Corp., Westborough, MD) was used to pattern channels on a Si wafer. PDMS (Sylgard 184 elastomer kit, Dow Corning Corp., Midland, MI) was then poured onto the mold, and cured overnight at 65 °C. The channel features one large (1.50 mm) channel over the BPE ends, with two small channels (0.05 mm) that were used to refresh the solution between trials. A split PPF BPE design was used, with 1.00 mm x 1.00 mm electrode tips exposed in each reservoir. The electrodes were cleaned with isopropanol prior to being irreversibly sealed to the PDMS channels. After assembly, Ag paint was applied to the PPF leads, and allowed to dry in a 65 °C oven for 30 min. Cu tape was then attached to the Ag paint in order to get sufficient contact to the PPF.

Electrochemical characterization. A CH Instruments Potentiostat (750E Series, Austin, TX) was used for all measurements. All measurements were taken under quiescent conditions. The solutions were refreshed between each run by pumping solution in through both small channels at a rate of 5.0 μL/min for 2 min. The solutions were allowed to sit for an additional 2 min before another measurement was taken. In the experiments employing a BPE, Ag/AgCl (sat. KCl) (Bioanalytical System Inc., West Lafayette, IN) were placed in the large reservoirs of the microfluidic device and used as driving electrodes. The driving electrode in the sensing channel
was connected to both counter and reference leads of the potentiostat, while the driving electrode in the reporting channel was connected to the working electrode lead. The two ends of the split BPE were connected to a multimeter (DMM6500 Keithley, Cleveland, OH), equipped with a current collection program (Kickstart Instrument Control Software, Keithley, Cleveland, OH) that sampled the current every 0.01 s. In the 3-electrode configuration, one half of the split BPE design was used as the working electrode, and both a Pt wire and a Ag/AgCl (sat. KCl) electrode were placed in the large reservoir as counter and reference electrodes.

Microscope and smartphone imaging and processing. A microscope (Nikon AZ100, Nikon Co., Tokyo, Japan) and a sCMOS camera (Andor Zyla 4.2, Oxford Instruments, Abingdon, Oxfordshire, England) were used to collect luminescence images under dark room conditions. The exposure time was 50 ms (20 fps) and 4 × 4 binning was used. A Google Pixel 2 (Android 10, HD 720p video resolution) with an attachable 20X macro lens was also used to collect luminescence images under dark room conditions. The frame rate was 24 fps.

To process the luminescence data, all videos were converted into frame by frame images. MATLAB code (Mathworks, Natick, MA) was written to sum the intensity of each pixel in an area designated as the electrode in each frame, with background subtraction. MATLAB code was also written to identify the amplitude of each peak in light intensity.

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Keywords: alternating current voltammetry, bipolar electrode, electrochemiluminescence, smartphone imaging

References


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**Scheme 1.**

**a)** Top view illustration of a BPE positioned between two microfluidic channels. A sensing reaction (BPE cathode) is coupled to a reporting reaction (BPE anode). A smartphone camera captures the luminescence at the BPE anode, while an ammeter monitors the current through the BPE. The amplitude of these signals is then calculated and compared. **b)** Side view of the closed split BPE configuration in a microfluidic device. **c)** Diagram illustrating an interfacial potential difference between the solution and each end of the closed BPE. Opposing ends of the BPE facilitate paired reactions (ferricyanide reduction and Ru(bpy)$_3^{2+}$ and TPA co-oxidation).
Scheme 2. a) Image of the split BPE microfluidic device with Ag/AgCl driving electrodes dipped into fluid reservoirs. b) Image obtained of BPE tip with smartphone camera with lens adaptations (channels outlined). One frame of the video obtained with the smartphone camera while performing ACV of the ECL reaction in both the c) 3-electrode and d) BPE configurations. In the BPE configuration, the ECL reaction at the BPE anode is coupled to ferricyanide reduction.
Scheme 3. a) Representation of linear sweep voltammograms of ferricyanide reduction (left) and ECL mixture co-oxidation (right). The markers located along the wave for ferricyanide reduction indicate current at a potential below $E_{1/2}$ (yellow), at $E_{1/2}$ (green), and larger than $E_{1/2}$ (purple). The corresponding markers on the oxidation wave depict currents that are equal in magnitude. b) Representation of the raw current response from ACV of ferricyanide (left) and ECL (right). The colored brackets indicate the amplitudes for the three cases described in (a). c) Representation of the raw luminescence response observed at the BPE anode during ACV of ferricyanide coupled to the ECL reaction. The three cases from (a) are indicated with brackets depicting the amplitude of the luminescence. d) Representation of the amplitude of the luminescence from (c) plotted against $\Delta E_{\text{tot}}$. The three cases from (a) are again depicted as markers.
Figure 1. Response of ECL reaction to ACV in a 3-electrode configuration. Luminescence data was obtained with a smartphone camera (24 fps). Raw luminescence ($I$) response obtained over time to ACV at a) 1.0 Hz and c) 5.0 Hz. Average amplitude of the current density ($J$, blue line) obtained from 3 devices, with corresponding average amplitude of luminescence ($L$, red line) for ACV at b) 1.0 Hz and d) 5.0 Hz, plotted against the applied DC step potential.
Figure 2. Average ($n = 3$) current density ($j$, blue triangles) and average ($n = 3$) luminescence response ($I$, red squares) obtained from a potential step ($E = 1.2$ V) applied to the driving electrode in the split BPE configuration with several distinct concentrations of ferricyanide as the sensing solution. At low concentrations reduction of ferricyanide limits the current density and luminescence. Therefore, a positive linear relationship is obtained for the two responses as the concentration of ferricyanide increases. At high concentrations of ferricyanide (around 10.0 mM), the ECL reporting reaction becomes limiting and therefore the current density and luminesce plateau.
Figure 3. Response obtained from 1.0 Hz ACV applied to 3.0 mM ferricyanide sensing solution paired with ECL reaction solution on BPE configuration. a) Raw current density ($j$) obtained over time from the ammeter connecting the two ends of the split BPE. b) Raw luminescence response ($l$) obtained over time from the smartphone camera. c) Amplitude of the current density ($J$, blue solid line) and luminescence ($L$, red dashed line) from (a).
Figure 4. Average ($n = 3$) peak amplitude of the alternating current density ($J_p$, blue triangles) obtained from several distinct concentrations of ferricyanide on the split BPE microfluidic device with a) 1.0 Hz and b) 5.0 Hz frequencies, compared with the simultaneously obtained peak amplitude of the alternating luminescence ($L_p$, red squares).
Graphical Abstract. Electrochemiluminescence is utilized to report the current obtained during alternating current voltammetry performed at a bipolar electrode. The resulting signal is captured by a smartphone camera, and the amplitude of this oscillating signal is plotted to obtain a luminescence voltammogram. This paper evaluates the utility of this method for sensing in a point-of-need setting.