Steady-State Voltammetry of a Microelectrode in a Closed Bipolar Cell

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ABSTRACT: Here we report the theory and experimental study of the steady-state voltammetric behavior of a microelectrode used as a limiting pole in a closed bipolar electrochemical cell. We show that the steady-state voltammetric response of a microelectrode used in a closed bipolar cell can be quantitatively understood by considering the responses of both poles in their respective conventional two-electrode setups. In comparison to a conventional electrochemical cell, the voltammetric response of the bipolar cell has a similar sigmoidal shape and limiting current; however, the response is often slower than that of the typical two-electrode setup. This leads to a broader voltammogram and a decreased wave slope, which can be somewhat misleading, causing the appearance that the process being studied is irreversible when it instead can be a result of the coupling of two reversible processes. We show that a large limiting current on the excess pole would facilitate the observation of a faster voltammetric response and that both redox concentration and electrode area of the excess pole affect the wave shape. Both factors should be maximized in electroanalytical experiments in order to obtain fast voltammetric responses on the main electrode of interest and to detect quick changes in analyte concentrations.

$$E = (E_a^{\circ\prime} - E_c^{\circ\prime}) + \frac{RT}{F}\left\{\frac{1}{n_a}\ln\left(\frac{i}{i_{ss}^a - i_a}\right) - \frac{1}{n_c}\ln\left(\frac{i_{ss}^c - i_c}{i_c}\right)\right\}$$
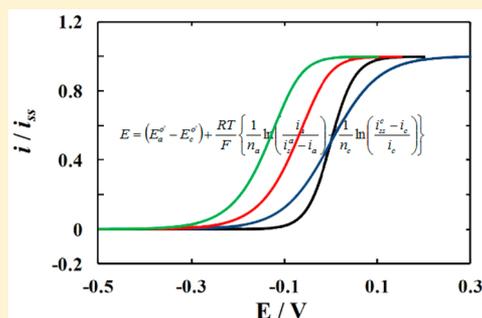
Bipolar electrochemistry has emerged as a useful tool for a number of important analytical applications,[1] such as electroanalysis,[2−7] separation,[8] preconcentration,[9−11] and neurochemical sensing using carbon-fiber microelectrodes.[12] In addition, bipolar electrochemistry mechanisms have been utilized in industrial processes for many decades.[13,14] Other recent applications of bipolar electrochemistry include synthesis and manipulation of nanomaterials[15−18] and fast screening of electrocatalysts.[19] A unique aspect of bipolar electrochemistry is that it uses an electrode with opposite polarity on two ends to allow for direct coupling of two different redox reactions. In a conventional two- or three-electrode electrochemical cell, one focuses primarily on electrochemical reactions at the working electrode. The reference and counter electrodes function only to provide a reference potential and balance the current on the working electrode. The counter electrode is usually sufficiently large so that it does not limit the Faradaic process on the working electrode. A bipolar electrochemical cell, on the other hand, involves two separate electrochemical interfaces coupled directly on a bipolar electrode (BPE). A pair of driving electrodes (usually much greater than the bipolar electrodes) supplies the voltage needed to drive the electrochemical reactions on the BPE. The overall electrochemical response of the BPE could thus be limited by both poles.

Electroanalytical studies involving BPEs have mainly been carried out using open BPEs. A typical open BPE cell uses a microfluidic channel to host a conductive microelectrode. A voltage bias is applied along the length of the microchannel from a pair of driving electrodes allowing two coupled electrochemical reactions to occur at each of the poles of the BPE. Open BPEs have been especially useful for performing wireless electrochemistry in a microfluidic environment.[1] We[20] and others[21,22] have recently discussed series-coupled electrochemical reactions on closed BPEs. As shown in Scheme 1a, a closed BPE cell contains two separate compartments connected via a metallic wire electrode. The electrochemical current on the closed BPE can be directly measured due to the lack of the microchannel and ionic current path. A closed BPE is analogous to two electrochemical cells connected in series, as depicted in Scheme 1b. In this configuration, the Faradaic reaction occurring on the disk electrode is electrochemically coupled to the reaction on the large cylinder electrode. By our convention we refer to the pole where the limiting process occurs as the limiting pole and the other as the excess or coupling pole.[20]

As an important application of a closed bipolar setup, one can make direct electrochemical current−voltage recordings on a microelectrode without the need to make direct electrical wire contact to the electrode. This is, in many cases, preferable to significantly simplify experimental procedures. For example, one can use a bipolar setup to measure the electrochemical response of single nanowires[20] and their arrays prepared in an insulating membrane. Additionally, carbon-fiber microelectrodes (CFEs),[23−25] when a salt solution is used to establish electrical continuity, use a closed bipolar mechanism to detect fast changes in catecholamine concentration.[20]

Although a microelectrode can be used in a closed bipolar setup to make direct electrode recordings, its electrochemical

Scheme 1. Schematic of (a) a Closed Bipolar Electrode Cell and (b) a Series-Coupled Bipolar Cell Containing a Disk Microelectrode in One Compartment and a Cylindrical Microelectrode in the Other Compartment[a]
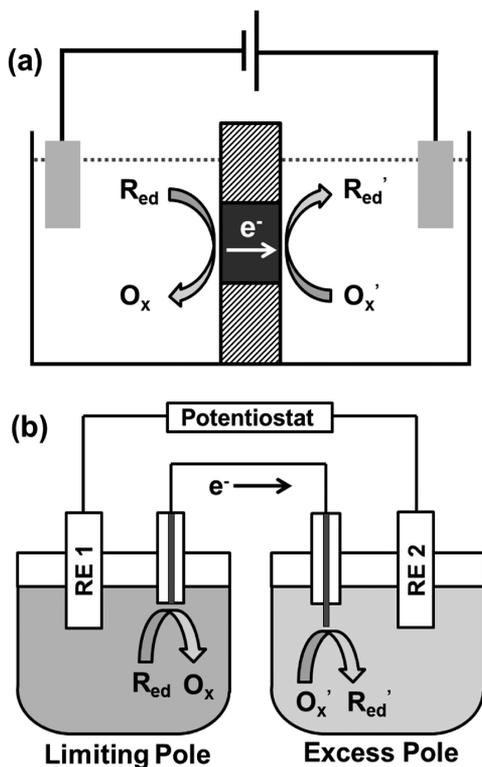
[a]The working electrode lead from the potentiostat is connected to reference electrode 2 (RE2) and the reference lead to reference electrode 1 (RE1). The anodic pole is limiting and the cathodic pole is in excess.

behavior can be quite different from its "normal" behavior in a two- or three-electrode setup. For example, in a previous study we have shown that the voltammetric response of a microelectrode in a closed bipolar cell is related to the properties of the excess pole with a shift in the response as a function of the difference in formal potentials of both poles. Additionally, there is a clear dependence of the half-wave potential on the redox concentration at and relative size of the excess pole.[20] However, in this previous report we did not reach a quantitative understanding of this relationship. In order to fully extend the potential use of BPEs for fundamental electrochemical studies as well as key bioanalytical applications, it becomes critical to develop a more comprehensive understanding of the steady-state voltammetric response of a microelectrode in a closed bipolar setup.

In this work, we discuss the theory to quantitatively describe the steady-state voltammetry of closed bipolar microelectrodes. We focus our attention on the steady-state voltammetry of a microelectrode used as a limiting pole of a BPE and how it is affected by the processes occurring at the excess pole. We show that the voltammetric response can be readily expressed by considering the individual responses of both poles separately in conventional two- or three-electrode configurations and that the wave shape depends strongly on the ratio of the limiting currents of both individual poles. We have also provided experimental results to support our theoretical prediction. Additionally, we discuss methods to obtain a fast and nearly

reversible voltammetric response from a microelectrode when used in a closed bipolar electrochemical configuration. These methods could be useful for improving performance of microscale BPEs such as CFEs routinely used for quantitative analysis in biological systems.

■ THEORY

In order to understand the voltammetric response of a microelectrode in a closed bipolar cell, it is beneficial to first consider the steady-state response of individual poles. Under simple mass-transport control, the reversible voltammetric response of a microelectrode in a conventional two-electrode cell can be readily expressed by the following equation[26]

$$E = E^{\circ\prime} + \frac{RT}{nF} \ln \frac{D_O}{D_R} - \frac{RT}{nF} \ln\left(\frac{i_{ss} - i}{i}\right) \tag{1}$$

where E and $E^{\circ\prime}$ are the applied electrode potential and the formal potential for the simple n-electron redox reaction $R_{ed} \rightarrow O_x + ne^-$, R is the gas constant, T is the absolute temperature, n is the number of electrons transferred per redox molecule, D_R and D_O are the diffusion coefficients for the redox species R_{ed} and O_x, respectively, i is the Faradaic oxidation current at voltage E, and i_{ss} is the steady-state limiting current. In our experiments the microelectrodes have inlaid disk geometry, and the limiting current is given by[27]

$$i_{ss} = 4nFDC^*r \tag{2}$$

where C^* is the concentration of redox species in the bulk and r is the radius of the microelectrode. Assuming the diffusion coefficients of the R_{ed} and O_x are approximately the same, eq 1 is further simplified as

$$E = E^{\circ\prime} - \frac{RT}{nF} \ln\left(\frac{i_{ss} - i}{i}\right) \tag{3}$$

A closed bipolar electrode allows one to couple two electrochemical reactions such that the Faradaic currents on the anodic pole and the cathodic pole have the same magnitude and opposite sign due to electroneutrality. Additionally, the overall bias voltage across the BPE is equal to the difference in the applied potentials on both poles.[20] This voltage can thus be expressed by combining versions of eq 3 for both cathodic and the anodic poles

$$\begin{aligned} E &= E_a - E_c \\ &= (E_a^{\circ\prime} - E_c^{\circ\prime}) + \frac{RT}{F}\left\{ \frac{1}{n_a} \ln\left(\frac{i_a}{i_{ss}^a - i_a}\right) \right. \\ &\quad \left. - \frac{1}{n_c} \ln\left(\frac{i_{ss}^c - i_c}{i_c}\right) \right\} \end{aligned} \tag{4}$$

where i_{ss}^a and i_{ss}^c are the limiting currents on the anodic pole and the cathodic pole, and $E_a^{\circ\prime}$ and $E_c^{\circ\prime}$ are the formal potentials for the reactions on the anodic and cathodic poles, respectively. *Here, we define the anodic current as positive and cathodic current as negative.* Because of electroneutrality in a closed bipolar cell, the magnitude of the oxidation current on the anodic pole should be equal to that of the reduction current on the cathodic pole, or, $i = i_a = -i_c$. By further assuming both reactions are one-electron processes, eq 4 becomes

$$E = (E_a^{\circ\prime} - E_c^{\circ\prime}) + \frac{RT}{F}\left\{\ln\left(\frac{i}{i_{ss}^a - i}\right) + \ln\left(\frac{-i}{i_{ss}^c + i}\right)\right\} \quad (5)$$

Equation 5 now shows a more complicated relationship between the total current i and the voltage across the bipolar electrode, which is closely dependent on the limiting currents of both poles. Several important conclusions can be drawn from eq 5. First, the limiting current on the bipolar electrode will be limited by the pole with smaller limiting current. If i_{ss}^a is smaller than $-i_{ss}^a$, the anodic pole will be the limiting pole and the limiting current of the BPE will be i_{ss}^a. Furthermore, $E_{1/2}$ can be derived from eq 5

$$E_{1/2} = (E_a^{\circ\prime} - E_c^{\circ\prime}) - \frac{RT}{F}\ln\left(-2\left(\frac{i_{ss}^c}{i_{ss}^a}\right) - 1\right) \quad (6)$$

Equation 6 indicates that the $E_{1/2}$ not only depends the difference of the formal potentials but is also closely related to the ratio of the limiting currents. The greater the $(-i_{ss}^c/i_{ss}^a)$, the more the $i-V$ response would shift negatively on the potential axis.

Additionally, the steady-state $i-V$ response of a microelectrode in a closed bipolar setup can now be predicted from eqs 4 and 5. Let us first consider a case where we have a simple $1e^-$ oxidation process, $R_{ed} \rightarrow O_x + 1e^-$, coupled to its corresponding reduction process. Since they are complementary reactions, their formal potentials are the same. As such, eq 5 becomes

$$E = \frac{RT}{F}\left(\ln\left(\frac{i}{i_{ss}^a - i}\right) + \ln\left(\frac{-i}{i_{ss}^c + i}\right)\right) \quad (7)$$

Figure 1a shows three predicted $i-V$ responses assuming that the limiting current on the cathodic pole is 1, 10, and 100 times of that on the anodic pole. Also shown in Figure 1a is the $i-V$ response of the anodic pole in a conventional two-electrode setup. This $i-V$ response has been generated assuming a 0 V formal potential for simplicity and easy comparison of the bipolar $i-V$ responses. All the current in Figure 1 has been normalized to the limiting current on the anodic pole for easy comparison.

One can immediately see that when compared to the $i-V$ response in a conventional two-electrode setup, all bipolar $i-V$ responses have similar sigmoidal shape. *However, the bipolar responses clearly show a slower current increase with voltage.* The 1:1 current ratio gives the slowest current increase and requires a higher applied voltage to reach steady state. We believe that this apparent wave broadening or slower $i-V$ response is solely due to the additional energy (or voltage) requirement to drive the second Faradaic reaction on the excess pole. The higher the cathodic limiting current, the faster the current increases with voltage. We can anticipate that when the cathodic steady-state limiting current is much higher than that on the anodic pole, the overall $i-V$ response would resume the reversible shape of the CV in the conventional two-electrode setup. Figure 1a also shows a clear negative shift of the $E_{1/2}$ of the bipolar response as the ratio of the limiting currents increases, which is in agreement with our previous observation.[20]

In order to more quantitatively understand the change in the shape of the bipolar $i-V$ response, we have generated the E vs $\log[i/(i_{ss} - i)]$ plots of the bipolar electrode and that of the conventional setup. Oftentimes a plot of E vs $\log[i/(i_{ss} - i)]$ is
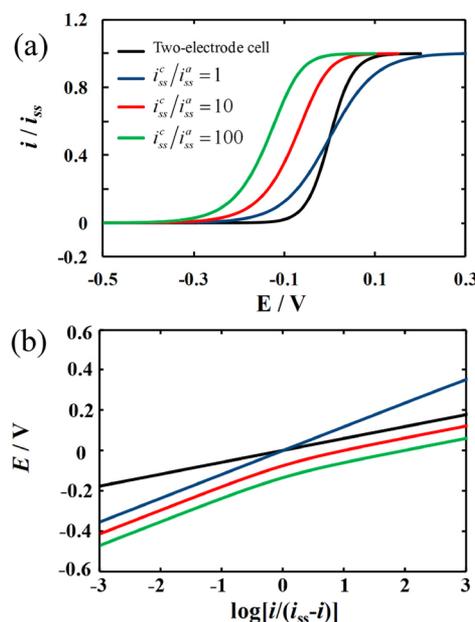
Figure 1. (a) Theoretical $i-V$ responses of a microelectrode in a two-electrode cell (black) and in a closed bipolar electrode setup when coupled to a cathodic pole. The blue, red, and green curves correspond to when the ratio of limiting currents, $-i_{ss}^c/i_{ss}^a$, are 1, 10, and 100, respectively. The formal potentials of the oxidation reaction on the microelectrode and the cathodic reaction on the coupling electrode have been assumed to be 0 V. The voltage for the bipolar setup is the voltage applied across the bipolar electrode and the voltage for the two-electrode setup is the voltage applied on the microelectrode with respect to the reference electrode. (b) A plot of the voltage applied across the bipolar electrode (for the blue, red, and green curves) as a function of the $\log[i/(i_{ss} - i)]$ for the $i-E$ curves given in part a.

prepared to show whether a process is reversible or not on a microelectrode.[26] If this plot is linear with a slope equal to $59.2/n$ mV, the process is said to be reversible. For a nonlinear plot with a slope greater than $59.2/n$ mV, the system is typically considered quasireversible or irreversible. The conventional direct connect configuration in Figure 1b shows a linear relationship with a slope of 59.2 mV as expected. On the other hand, for the bipolar setup, a linear relationship is obtained only when both poles have the same limiting current, in which case the slope of the curve is 118 mV. When the cathodic pole has a greater limiting current than the anodic pole, a nonlinear relationship is obtained between E and the $\log[i/(i_{ss} - i)]$, as predicted from eqs 4 and 5. The slope of the line at $E_{1/2}$ is a function of the ratio $-i_{ss}^c/i_{ss}^a$ and is between 59 and 118 mV. We can anticipate that when this ratio goes to infinity, the slope of the line would approach 59 mV at half-wave potential.

This increased slope may be mistakenly understood to be an indication of irreversibility. Instead it is the result of directly coupling two reversible processes and is not itself indicative of any kinetic limitations. An alternative approach to determine the reversibility of an electrochemical process is to apply the Tomeš criterion of reversibility, which states that for a reversible charge transfer the difference in quartile potentials, $|E_{3/4} - E_{1/4}| = 56.4/n$ mV, where $E_{3/4}$ and $E_{1/4}$ refer to the three-quarter- and one-quarter-wave potentials, respectively.[26] From eq 5 we can derive the modified Tomeš criterion of reversibility for two coupled electrochemical reactions at a BPE to be

$$|E_{3/4} - E_{1/4}| = \frac{RT}{F} \ln\left(27\left(\frac{i_{ss}^c + 0.25i_{ss}^a}{i_{ss}^c + 0.75i_{ss}^a}\right)\right) \qquad (8)$$

This will allow for a more complete description of processes occurring at a microelectrode when used in a closed bipolar cell configuration as a tool for electrochemical analysis. By applying this criterion to the simulated results, values of 113, 86, and 85 mV for 1:1, 10:1, and 100:1 are calculated, respectively.

■ EXPERIMENTAL SECTION

Reagents and Chemicals. Ferrocene (Fc, Fluka Analytical), dopamine hydrochloride (DA, Sigma-Aldrich), potassium ferricyanide $[K_3Fe(CN)_6$, Sigma-Aldrich], potassium ferrocyanide $[K_4Fe(CN)_6$, Fluka], potassium chloride (KCl, J. T. Baker), hexaamineruthenium(III) chloride $[Ru(NH_3)_6Cl_3$, Aldrich], tetra-n-butylammonium hexafluorophostphate (TBAPF$_6$, Aldrich), perchloric acid (HClO$_4$, Aldrich), and reagent grade acetonitrile (MeCN, Aldrich) were all used without further purification. All aqueous solutions were prepared using $\geq$18 MΩ cm water from a Barnstead NanoPure purification system (Thermo Scientific). Oxygen reduction reactions were carried out in a 0.1 M KOH (Mallinckrodt) solution that had been bubbled with O$_2$ for a minimum of 20 min.

Electrode Fabrication. Metal microelectrodes were fabricated by encapsulating wire in a glass capillary. Pt and Au microwires with a diameter of 25 μm (99.95%, hard, Alfa Aesar) were partially sealed in a borosilicate capillary (o.d. = 2.0 mm, i.d. = 1.0 mm, Sutter Instrument Co.) using a vacuum and hydrogen flame. Electrical contact was made by connecting the unsealed portion of the wire to a 150 μm tungsten wire with conductive silver paste (DuPont). The sealed portion of the wire was exposed with regular sandpaper and an alumina suspension on a wet polishing cloth. Carbon-fiber microelectrodes were fabricated according to our previously published methods.[28] Briefly, a 5-μm-diameter carbon fiber was aspirated into a borosilicate glass capillary (o.d. = 1.2 mm, i.d. = 0.69 mm, Sutter Instrument Co.) and pulled with a micropipet puller (Model P-97, Sutter Instrument Co.). Afterward, the pulled tip was sealed with epoxy (Epoxy Technology) and beveled to 45°. For a direct connect carbon fiber electrode, electrical contact to the carbon fiber was achieved with conductive silver paste from inside the pulled glass capillary.

Cyclic Voltammetry. All current–voltage responses were measured using a Chem-Clamp voltmeter/amperometer (Dagan) and a PAR 175 (Princeton Applied Research) universal function generator. The potentiostat was interfaced to a Dell computer through a PCI-6251 data acquisition board (National Instruments) via a BNC-2090 analog breakout box (National Instruments). The current–voltage data was recorded and analyzed using an in-house written virtual instrumentation with LabView 8.5 (National Instruments). For simplicity, a direct-connect setup refers to a traditional one-compartment two-electrode cell with one working electrode and a Ag/AgCl reference electrode (Bioanalytical Sciences Inc.). A closed BPE cell was constructed by connecting two cells in series with two working microelectrodes connected.[20] Two Ag/AgCl electrodes were used to supply the driving voltage bias of the closed BPE cell.

■ RESULTS AND DISCUSSION

Steady-State Voltammetry of a Microelectrode in a Closed Bipolar Cell. Our theory has shown that when a microelectrode is used as the limiting pole in a closed bipolar setup, it would show a sigmoidal shape current–voltage response. Compared to its voltammetric response in a conventional two-electrode setup, the bipolar response has the same limiting current. However, the $E_{1/2}$ and the shape of the voltammetric response are strongly dependent on the property of the excess pole. In general, the bipolar response is slower than the response in a conventional two-electrode cell, as evident by the broadening of the wave. The distortion is however unrelated to electron-transfer kinetics and is simply due to electrochemical coupling in a bipolar cell.

Figure 2 displays the voltammetric responses of a 25-μm-diameter Au disk electrode in an aqueous solution of 1 mM
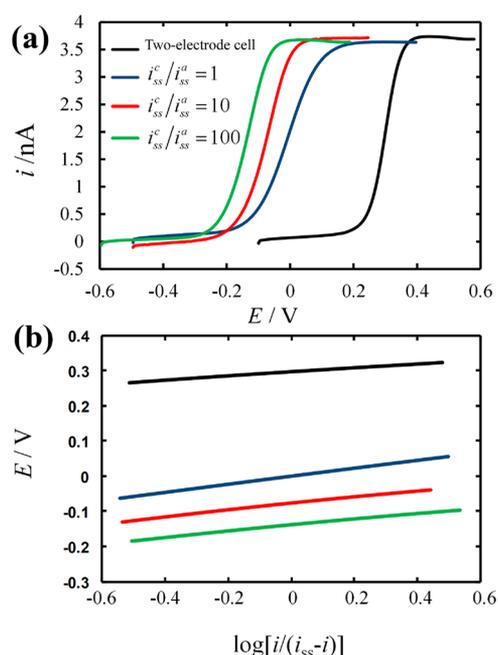
Figure 2. (a) The i–V responses of a 25-μm-diameter Au disk microelectrode in a solution of 1 mM Fe(CN)$_6^{4+}$ and 3 M KCl in a two-electrode setup (black curve) and a closed bipolar cell when coupled to a second 25-μm-diameter Au disk electrode as a cathodic pole. The cathodic pole was placed in a 3 M KCl solution containing Fe(CN)$_6^{3-}$ of various concentrations: 1 mM (blue curve), 10 mM (red curve), and 100 mM (green curve). The scan rate was 20 mV/s for all scans. The voltage for the bipolar setup is the voltage applied across the bipolar electrode, as illustrated in Scheme 1b, and the voltage for the two-electrode setup is the voltage applied on the microelectrode with respect to a Ag/AgCl reference electrode. (b) A plot of the voltage applied across the bipolar electrode (for the blue, red, and green curves) as a function of the $\log[i/(i_{ss} - i)]$ for the i–E curves given in part a. Only voltages between $E_{1/4}$ and $E_{3/4}$ are plotted in part b.

ferrocyanide and 3 M KCl in three different setups. The black curve is the i–V response of the electrode in a two-electrode cell. A nice sigmoidal shape response is obtained for the oxidation of Fe(CN)$_6^{4+}$. The limiting current is 3.69 nA, in good agreement with the prediction of 3.66 nA from eq 2. The blue, red, and green curves are i–V responses recorded from the same electrode in a closed bipolar cell. A second Au disk electrode of the same size is used as a cathodic pole. In this

case, the cathodic pole is placed in an aqueous solution containing 1 mM ferricyanide and 0.1 M KNO_3 (blue curve). Since $Fe(CN)_6^{3-}$ has the same concentration and roughly the same diffusion coefficient as $Fe(CN)_6^{4-}$, the cathodic pole would yield a reduction limiting current equal to the oxidation limiting current on the anodic Au electrode (i.e., the electrode of interest), or $-i_{ss}^c/i_{ss}^a = 1$. One can clearly see that the $i-V$ response (blue) has roughly the same limiting current as that recorded in the two-electrode setup (black). However, the bipolar response is clearly slower than that of the two-electrode setup. This result is in good agreement with prediction from Figure 1a. The slower $i-V$ response in the bipolar setup can be qualitatively understood by the additional energy/voltage requirement to drive the electrochemical process on the cathodic pole. The higher the redox concentration in the cathodic pole, the less voltage is needed to drive the same amount of current on the cathodic pole. In a second experiment, the cathodic pole is placed in a 10 mM $Fe(CN)_6^{3-}$ solution (red curve). This would correspond to a situation in which the cathodic limiting current is roughly 10 times greater than the anodic pole, or $-i_{ss}^c/i_{ss}^a = 10$. Compared to the blue curve, the red $i-V$ response indeed becomes much faster due to the increase in the cathodic limiting current. An even stronger effect is observed when the cathodic Au electrode is placed in a 100 mM $Fe(CN)_6^{3-}$ solution (green curve). Additionally, there is a clear negative shift in the $E_{1/2}$ as the concentration of $Fe(CN)_6^{3-}$ is increased, which is in good agreement with eq 6 and results in Figure 1.

We have plotted the voltage across the bipolar electrode with respect to the logarithm of the limiting current ($\log[i/(i_{ss} - i)]$), as shown in Figure 2b. Also shown is the same plot for the electrode in the two-electrode setup (the black curve). The two-electrode setup yields a linear curve with a slope of 59.3 mV as expected, indicating a reversible response. Conversely, the blue curve is the plot for the same electrode in the bipolar setup with $-i_{ss}^c/i_{ss}^a = 1$. The blue curve is also found to be linear with a slope of 113.5 mV, in agreement with prediction in Figure 1b. The red and green curves are the plots of the same electrode in the bipolar setup corresponding to when $-i_{ss}^c/i_{ss}^a$ is ~10 and ~100, respectively. These are nonlinear plots, and the slopes at $E_{1/2}$ are 93.9 and 83.3 mV. The values obtained for |$E_{3/4} - E_{1/4}$| are shown in Table 1 and are in agreement with the derived modified Tomeš criterion of reversibility. In summary, our experimental results have confirmed the theoretical prediction.

Both theory and experimental results have revealed that when a microelectrode is used in a bipolar setup, its

voltammetric response may change significantly, depending on the properties of two coupling poles. Most significantly, a microelectrode shows a slower $i-V$ response in a bipolar setup than its corresponding response in a conventional two- or three-electrode cell. This sluggish voltammetric behavior is a result of electrochemical coupling and extra voltage requirement to drive complementary current at the other pole. A greater limiting current on the coupling pole could lower this voltage requirement. Therefore, it could likely facilitate the observation of a faster voltammetric response.

Is "Reversible" Voltammetric Behavior Obtainable in a Bipolar Cell? A bipolar mechanism can greatly simplify the experimental setup in certain experiments involving ultra-microelectrodes and nanoelectrodes by eliminating direct wire contacts to the electrodes. For example, carbon-fiber micro-electrodes use a salt solution to establish electrical contact, which makes it convenient to quickly change electrodes during single-cell measurements. However, the electrochemical coupling between the two poles could complicate the analysis of the voltammetric behavior of a micro- or nanoelectrode by slowing down its $i-V$ response. Although the limiting current is unlikely affected, the shape of the voltammetric response could depend strongly on the condition of the coupling pole. This could have significant impacts for electroanalytical applications where the shape of the voltammetric response is important. For example, the shape of the steady-state voltammogram of very small nanoelectrodes can be used to analyze heterogeneous electron-transfer kinetics.[29,30] Additionally, the sluggish voltammetric response of microelectrodes in a bipolar setup may cause increased uncertainty in certain electroanalytical sensing experiments, such as detection of electroactive neurotransmitters in single-cell measurements. It is reasonable to believe that a faster voltammetric response would be preferable in almost all electroanalytical experiments involving the use of micro-electrodes and nanoelectrodes and a bipolar mechanism to establish electrical contact.

It is thus important to explore possible methods to obtain a nearly "reversible" voltammetric response in a closed bipolar setup. Because the slow voltammetric response is a result of the additional voltage requirement from the coupling pole, we believe that a greater limiting current at the coupling pole could facilitate the observation of a nearly "reversible" voltammetric response. Since the limiting current on the coupling pole depends on the concentration of the redox species and electrode size and both factors are relatively easy to adjust, we set out experiments to obtain fast voltammetric responses by changing redox concentrations and greatly increasing the size of the excess pole.

Figure 3 shows a series of $i-V$ responses of a 25-μm-diameter Pt disk microelectrode in a closed bipolar setup in acetonitrile containing 50 μM Fc and 0.1 M $TBAPF_6$. A second 25-μm-diameter Pt disk electrode is used as the excess cathodic pole and is placed in an aqueous solution containing 3 M KCl and $Fe(CN)_6^{3-}$ of various concentrations. This electrochemical cell configuration is similar to the one shown in Scheme 1b, except that the cathodic pole is a disk microelectrode. Here, the oxidation of Fc on the first Pt microelectrode is coupled to the $Fe(CN)_6^{3-}$ reduction on the cathodic pole, which also limits the overall $i-V$ response of the BPE. All the $i-V$ responses shown in Figure 3 have nearly the same limiting current due to Fc oxidation. The measured limiting current agrees well with prediction according to eq 2.

Table 1. A Comparison of the Wave Parameters Obtained from the Predicted $i-V$ Curves in Figure 1 and That Measured from Experimental $i-V$ Curves in Figure 2 for Different $-i_{ss}^c/i_{ss}^a$ Ratios

$-i_{ss}^c/i_{ss}^a$ (mV)	wave slope (mV)		|$E_{3/4} - E_{1/4}$| (mV)	
	measured	calcd	measured	calcd
1:1	113.5	118	111	113
10:1	93.9	86	87	86
100:1	83.3	85	79	85
two-electrode[a]	59.3	59.2	56	56.4

[a]The corresponding wave parameters for the two-electrode cell are also listed for comparison.

Figure 3. The $i-V$ responses of a 25-μm-diameter Pt disk electrode in acetonitrile containing 50 μM Fc and 0.1 M TBAPF$_6$. The oxidation of Fc was coupled to the reduction of ferricyanide of varying concentrations in a 3 M KCl solution on another 25-μm-diameter Pt microelectrode.

A general trend is observed in Figure 3: the Fc oxidation current increases much more quickly with voltage as the concentration of $Fe(CN)_6^{3-}$ increases. In the absence of $Fe(CN)_6^{3-}$, the oxidation of Fc is likely coupled to the reduction of soluble oxygen on the cathodic pole. A much slower $i-V$ response is observed due to a smaller reduction current of oxygen. The oxidation of Fc becomes much faster in the presence of $Fe(CN)_6^{3-}$. The higher the $Fe(CN)_6^{3-}$ concentration, the faster the current increases with voltage. Because the reduction current of $Fe(CN)_6^{3-}$ is roughly proportional to its concentration, the observed concentration effect on the $i-V$ response is analogous to the effect of the current ratio of two poles.

It is advantageous in a closed bipolar setup to use a high redox concentration on the coupling pole. However, the redox concentration is often limited in many conditions. For example, the concentration of saturated $Fe(CN)_6^{3-}$ is ~1.4 M at room temperature.[31] Since the limiting current on the coupling pole is also directly related to its size, it is in many conditions convenient to use a large coupling pole to obtain a fast voltammetric response on the limiting pole.

Figure 4 shows a series of $i-V$ responses of a 25-μm-diameter Pt disk microelectrode in acetonitrile containing 0.5 mM Fc and 0.1 M TBAPF$_6$. The Pt disk electrode has been coupled to a 25-μm-diameter 2-mm-length Pt wire electrode used as an excess pole. The Pt microwire was placed in three different aqueous solutions of 5 mM $Fe(CN)_6^{3-}$, 5 mM $Ru(NH_3)_6^{3+}$, and oxygen-saturated 0.1 M NaOH. The blue curve is the $i-V$ response of the same 25-μm-diameter Pt disk electrode in 0.5 mM Fc in a two-electrode setup for comparison. One can see that all $i-V$ responses in Figure 4 show very similar sigmoidal shape except for a noticeable shift in their $E_{1/2}$. The $i-V$ response shows the minimum shift in position when the disk electrode is coupled to the reduction of oxygen on the Pt wire. The greatest $E_{1/2}$ shift is observed when the disk electrode is coupled to the reduction of $Fe(CN)_6^{3-}$. The shift in $E_{1/2}$ can be qualitatively explained by considering the formal potentials of the redox species on both poles and the effect of the limiting current ratio. The formal potential of Fc oxidation is ~0.55 vs NHE.[26] When the disk electrode is coupled to oxygen reduction with a formal potential of ~−0.065 V vs NHE,[32] an $E_{1/2}$ around +0.61 V is anticipated. The purple $i-V$ response shows an $E_{1/2}$ of around 0.4 V, a negative shift of 0.21 V, which is caused by the large limiting current of oxygen reduction. Similar potential shifts have been
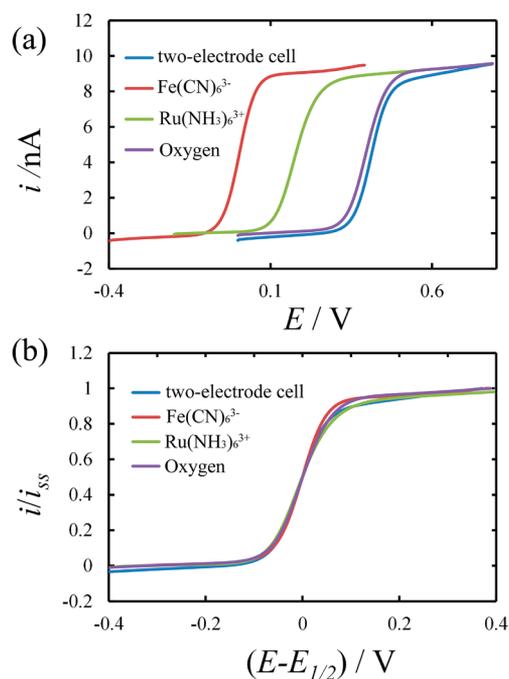
Figure 4. (a) The $i-V$ responses of a 25-μm-diameter Pt disk electrode in acetonitrile containing 0.5 mM Fc and 0.1 M TBAPF$_6$. The oxidation of Fc was coupled to the reduction of three different redox species, 5 mM $Fe(CN)_6^{3-}$, 5 mM $Ru(NH_3)_6^{3+}$, and saturated oxygen in 0.1 M NaOH on a 25-μm-diameter, 2-mm-length Pt wire. The $i-V$ response of the same electrode in a two-electrode cell is given as the blue curve for comparison. (b) An overlay plot of the normalized CVs in part a highlighting the change in wave shape.

observed in Figures 1 and 2. Similarly, the reduction of $Fe(CN)_6^{3-}$ has a formal potential of 0.36 V vs NHE,[26] which would result in an $E_{1/2}$ of the bipolar response around 0.2 V. It is observed in Figure 4a that the $E_{1/2}$ is around 0 V, a negative shift of ~0.2 V again caused by the large ratio of the limiting current. The reduction of $Ru(NH_3)_6^{3+}$ has a formal potential of ~0.1 V vs NHE,[26] which gives a predicted $E_{1/2}$ of around 0.45 V. However, the observed $E_{1/2}$ is around 0.2 V due to a negative shift of ~0.25 V.

Importantly, a quick examination of Figure 4a reveals that all $i-V$ responses are very similar in overall shape. Figure 4b shows all four curves superimposed on each other. There is hardly any differences observable between the two-electrode response and those of the bipolar setups in $Fe(CN)_6^{3-}$ and $Ru(NH_3)_6^{3+}$. To quantitatively describe differences in wave shapes, the wave slope and $|E_{3/4} - E_{1/4}|$ are shown in Table 2. The calculated values show a slight increase in wave slope and $|E_{3/4} - E_{1/4}|$ for the bipolar configurations, as expected. The fact that all $i-V$

Table 2. Wave Parameters Obtained from the $i-V$ Curves in Figure 4 for Fc Oxidation at a Pt BPE

coupling redox	wave slope (mV)	$|E_{3/4} - E_{1/4}|$ (mV)
K$_3$Fe(CN)$_6$	89.1	85
Ru(NH$_3$)$_6$Cl$_3$	84.9	81
oxygen	75.5	72
two-electrode[a]	68.1	65

[a]The corresponding wave parameters for the two-electrode cell are also listed for comparison.
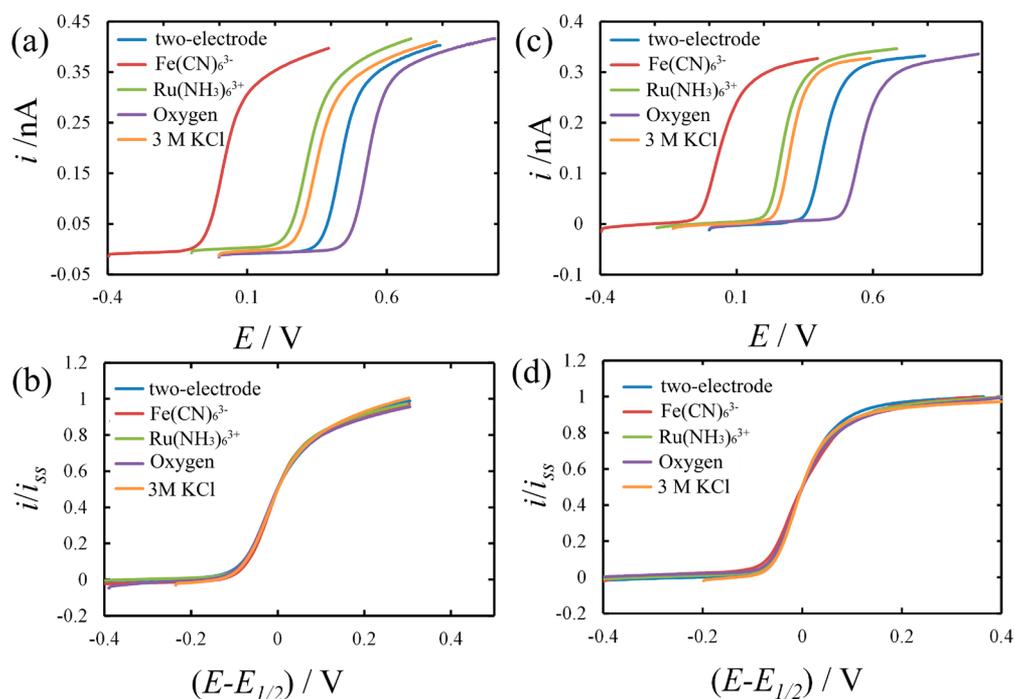
Figure 5. The $i-V$ responses of a 5-μm-diameter carbon-fiber disk electrode in a solution of (a) 50 μM Fc 0.1 M TBAPF$_6$ and (c) 100 μM dopamine coupled to a 5-μm-diameter, 2-mm-length carbon fiber in a solution containing different redox molecules including 5 mM Fe(CN)$_6^{3-}$, 5 mM Ru(NH$_3$)$_6^{3+}$, saturated oxygen in 0.1 M NaOH, and 3 M KCl. The CV response of the same disk electrode in a two-electrode cell is given for comparison. An overlay plot of the normalized CVs is given in part b for Fc and part d for dopamine to highlight the change in wave shape.

responses have similar shapes and no major distortion is observed in the bipolar setups could be due to the significantly larger Faradic current on the Pt wire electrode. A CV response of the Pt wire electrode in 5 mM Fe(CN)$_6^{3-}$ (not shown) shows a reduction current of ~2500 nA, which is roughly 275 times greater than the limiting current on the Pt disk. The results shown in Figure 4 are encouraging because they indicates that one can obtain a nearly reversible $i-V$ response, even in a bipolar setup when a microelectrode is coupled to a large excess pole. This is an important result, because it implies that when a bipolar setup is used in electroanalytical applications, one should use a large coupler electrode in order to more accurately examine the voltammetric properties of a microelectrode or to detect quick changes in analyte concentrations.

Due to the wide usage of carbon-based microelectrodes in bioanalytical sensing, it is important to verify the above conclusions with CFEs. As shown in Figure 5, similar observations have been obtained with carbon-fiber micro-electrodes. Figure 5a is a series of $i-V$ responses collected from a closed bipolar cell consisting of a 5-μm-diameter disk CFE in a 50 μM Fc solution containing 0.1 M TBAPF$_6$ and a CFE with an exposed 5-μm-diameter, 2-mm-length carbon fiber in varying reduction reactions. The $i-V$ response of the same disk CFE in a two-electrode cell is given as the blue curve, in which a direct electrical contact was made to the fiber from inside the glass capillary using silver epoxy. Figure 5b is the overlaid $i-V$ responses after correcting their $E_{1/2}$ shifts. One can see that all the $i-V$ responses in the bipolar setup have similar shapes and limiting currents, which are nearly indistinguishable from the response in the two-electrode setup.

In addition to using Fc oxidation as a model reaction, we have studied the oxidation of dopamine on a 5-μm-diameter CFE. Figure 5c shows a series of $i-V$ responses collected on a 5-μm-diameter CFE in 100 μM dopamine when coupled to the

reduction of several different redox species including Fe-(CN)$_6^{3-}$, Ru(NH$_3$)$_6^{3+}$, saturated oxygen in 0.1 M NaOH, and soluble oxygen in 3 M KCl. The resulting $i-V$ responses all show very similar shapes and are almost indistinguishable from the one collected in a two-electrode setup on the same electrode, as shown in Figure 5c,d. The wave slope and $|E_{3/4} - E_{1/4}|$ for the curves in Figure 5a,c are shown in Table 3 and demonstrate little variance between bipolar configurations. The results shown in Figure 5 have further confirmed that when a microelectrode is used in a bipolar setup, a large coupler electrode is needed to obtain nearly reversible voltammetric responses.

Table 3. Wave Parameters Obtained from the $i-V$ Curves in Figure 5 for the Oxidation of Fc and Dopamine at a Carbon Fiber Microelectrode Coupled to a Carbon Fiber Wire Electrode in Different Redox Solutions

redox species			
limiting pole	coupling pole	wave slope (mV)	$|E_{3/4} - E_{1/4}|$ (mV)
ferrocene	K$_3$Fe(CN)$_6$	69.2	66
	Ru(NH$_3$)$_6$Cl$_3$	68.1	65
	oxygen	70.8	67
	KCl	68.1	65
	two-electrode[a]	60.8	59
dopamine	K$_3$Fe(CN)$_6$	83.8	80
	Ru(NH$_3$)$_6$Cl$_3$	78.6	75
	oxygen	89.1	85
	KCl	83.8	80
	two-electrode[a]	78.6	74

[a]The corresponding wave parameters for the two-electrode cells are also listed for comparison.

CONCLUSION

We have discussed the theory and corresponding experiments to more fully understand the steady-state voltammetric response of a microelectrode used in a closed bipolar setup. Our theory shows that the voltammetric response is likely distorted when a microelectrode is used in a bipolar setup and is often slower than that in a conventional two-electrode setup. The slower response in the bipolar setup is likely due to additional voltage/energy requirement to drive the Faradaic reaction at the coupling pole. The shape of the bipolar voltammetric response depends on the ratio of limiting currents on both poles. A large Faradic current on the excess coupling pole facilitates the observation of a fast voltammetric response. This can be realized by increasing the redox concentration and the electrode size on the excess coupling pole.

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Notes

The authors declare no competing financial interest.

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