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Laboratories and Demonstrations

A Study of the Oxidation Pathway of Adrenaline by Cyclic Voltammetry: An Undergraduate Analytical Chemistry Laboratory Exercise

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…an interesting real-world laboratory problem, the electrochemical characterization of adrenaline, which implements this important technique.

An undergraduate laboratory exercise to be used in an undergraduate instrumental methods course is presented for the electrochemical characterization of oxidation products of adrenaline. An objective of this experiment is to use information obtained through cyclic voltammetry and simulation software to develop a qualitative understanding of the oxidation mechanism of adrenaline.

Introduction

The topic of electrochemistry is typically taught in junior- and senior-level undergraduate instrumentation courses. While there are a relatively large number of electrochemical laboratory exercises to complement such courses, there are few experiments utilizing one of the most common electrochemical methods—cyclic voltammetry. Thus, we have developed an

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interesting real-world laboratory problem, the electrochemical characterization of adrenaline, which implements this important technique.

Cyclic voltammetry is by far the most widely used electrochemical method. This potential-sweep method involves the linear application of a potential to a working electrode between two predetermined values by using a potentiostat (see Figure 1A). The resultant current is plotted versus the applied potential, and this plot is referred to as a voltammogram (see Figure 1B). The shape of the voltammogram is diagnostic of the electrode process. For a reversible electron-transfer process, the formal reduction potential ($E°'$) is directly calculated from the midpoint between the anodic and cathodic peak potentials. The theory of cyclic voltammetry is well established for many electrode reaction mechanisms, and a relatively straightforward description of this technique is found in various chemical education references [1–3]. These articles serve as the basis for the lecture material covered in our instrumentation course. Readers who are unfamiliar with the technique and terminology of cyclic voltammetry are referred to these articles for more information related to mechanistic data ascertained from cyclic voltammograms.

Electron-Transfer Mechanisms Coupled to Chemical Reactions

The power of cyclic voltammetry as an analytical tool lies in its ability to probe electron-transfer mechanisms that are coupled to chemical reactions. For a simple electron-transfer process, an electron-transfer reaction occurs directly at the surface of the working electrode as follows:

$$A_{ox} + n\,e^- \rightleftharpoons A_{red} \qquad (1)$$

If electron transfer is rapid (i.e. reversible), the concentrations of oxidized and reduced species at the electrode surface are established according to the Nernst equation:

$$E_{app} = E°' + (RT/nF) \log ([A_{ox}]/ [A_{red}]) \qquad (2)$$

where E_{app} is the potential applied to the working electrode. The formal equilibrium reduction potential ($E°'$) is the applied potential where the concentrations of A_{ox} and A_{red} at the electrode surface are the same. For a scan through a potential region much more negative than the formal equilibrium reduction potential ($E°'$), the oxidized species (A_{ox}) will essentially be reduced near the electrode surface. Likewise, this

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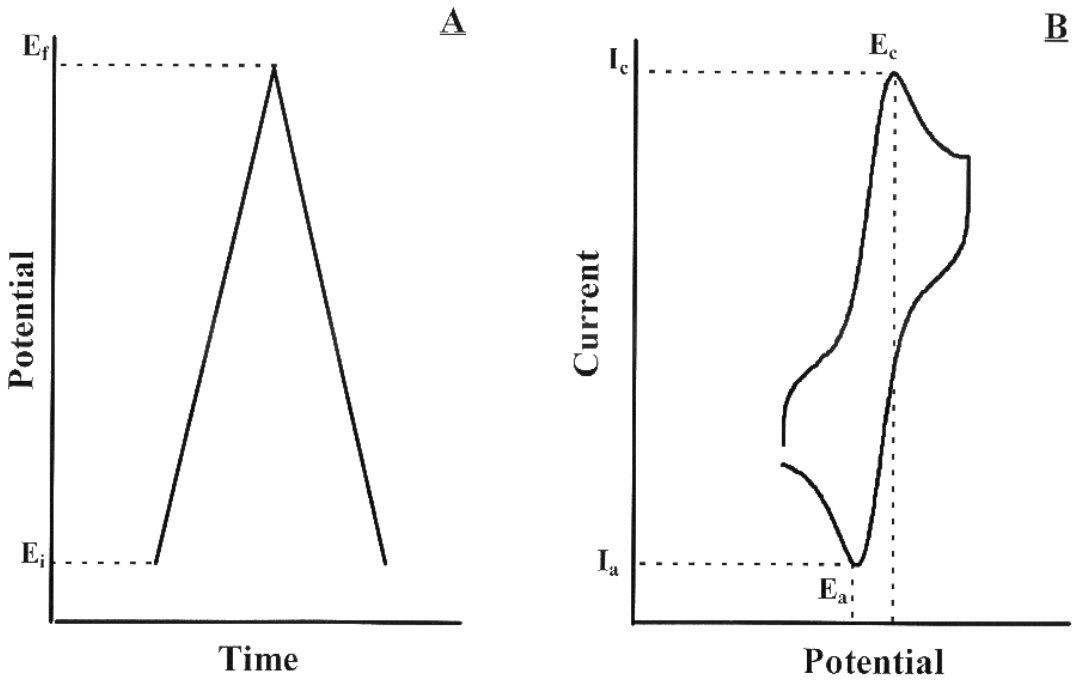

FIGURE 1. A) APPLIED POTENTIAL VERSUS TIME WAVEFORM FOR CYCLIC VOLTAMMETRY. E_I AND E_F ARE THE INITIAL AND FINAL APPLIED POTENTIALS, RESPECTIVELY. B). CHARACTERISTIC RESULTANT CURRENT VERSUS APPLIED POTENTIAL RESPONSE FOR A REVERSIBLE ELECTRON-TRANSFER MECHANISM. E_C = CATHODIC PEAK POTENTIAL, E_A = ANODIC PEAK POTENTIAL, I_C = CATHODIC PEAK CURRENT, AND I_A = ANODIC PEAK CURRENT.

species will be completely reoxidized at the electrode surface during the reverse scan at applied potentials much more positive than $E^{\circ\prime}$.

For an unstirred solution, the amount of A_{red} available near the electrode surface during the oxidative scan is determined by the rate of electron transfer for oxidation ($A_{red} \rightarrow A_{ox}$), the rate of diffusion of A_{red} away from the electrode surface, and the rate of any coupled chemical reaction which depletes the concentration of A_{red} (e.g., $A_{red} \rightarrow$ B). The concentration of A_{red} near the electrode surface will also be determined by the formal equilibrium reduction potential of A, and the equilibrium constant for the chemical step ($A_{red} \rightleftharpoons$ B). Furthermore, if the product from a chemical reaction is electroactive within the scanned potential range, then this new species will also give rise to a voltammetric wave (e.g., $B_{ox} + n\ e^- \rightleftharpoons B_{red}$). Thus, the appearance of additional voltammetric waves after an electrochemical step is expected for succeeding chemical reactions that generate electroactive products. Specific electron-transfer and chemical steps for a particular mechanism are designated by convention as E and C

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steps, respectively. For example, $A_{ox} \rightleftharpoons A_{red} \rightleftharpoons B$ would be referred to as an EC mechanism.

Recording voltammograms as a function of experimental conditions (e.g., scan rate, concentration, pH) is commonly done to probe electron-transfer mechanisms. For example, recording peak current as a function of scan rate provides a simple test for analyzing electron-transfer mechanisms that are coupled to chemical reactions. Voltammograms typically resemble the reversible case at high scan rates for a following a chemical reaction mechanism since little time has transpired for chemical conversion between forward and reverse scans. The ability to probe complex electron-transfer mechanisms through cyclic voltammetry has recently been greatly improved through the use of CV simulators (e.g., DigiSim, BAS, Lafayette, IN) [4]. Based on a proposed electron-transfer mechanism, data can be simulated for various values for heterogeneous rate constants associated with electron transfer ($k°$), formal reduction potentials ($E°'$), equilibrium constants for coupled chemical steps (K), and homogeneous rate constants associated with the forward chemical steps (k_f).[1] These simulated data are then compared to experimental results. DigiSim is particularly instructive since it also allows for students to visualize the changes in concentrations of various species in solution near the electrode surface.

The purposes of this laboratory exercise are to introduce students to cyclic voltammetry, to probe an electron-transfer reaction coupled to chemical steps, and to familiarize students with simulation software. This experiment involves the electrochemical characterization of adrenaline oxidation as a function of pH and scan rates. The oxidation of adrenaline under appropriate pH conditions results in the formation of additional electroactive species as discussed below. Students make solutions and perform the electrochemical experiments in the first laboratory session, and then use simulation software to reproduce experimental results in the subsequent laboratory session. Each of these exercises can be successfully completed within three hours.

[1] The rate constant for the reverse step (k_b) is automatically calculated from the forward rate constant and equilibrium constant.

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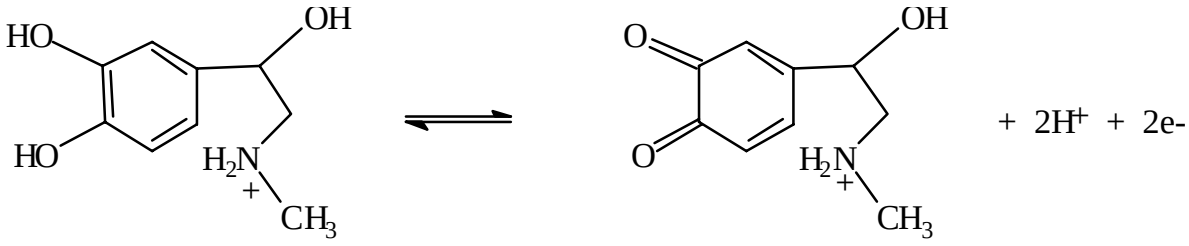
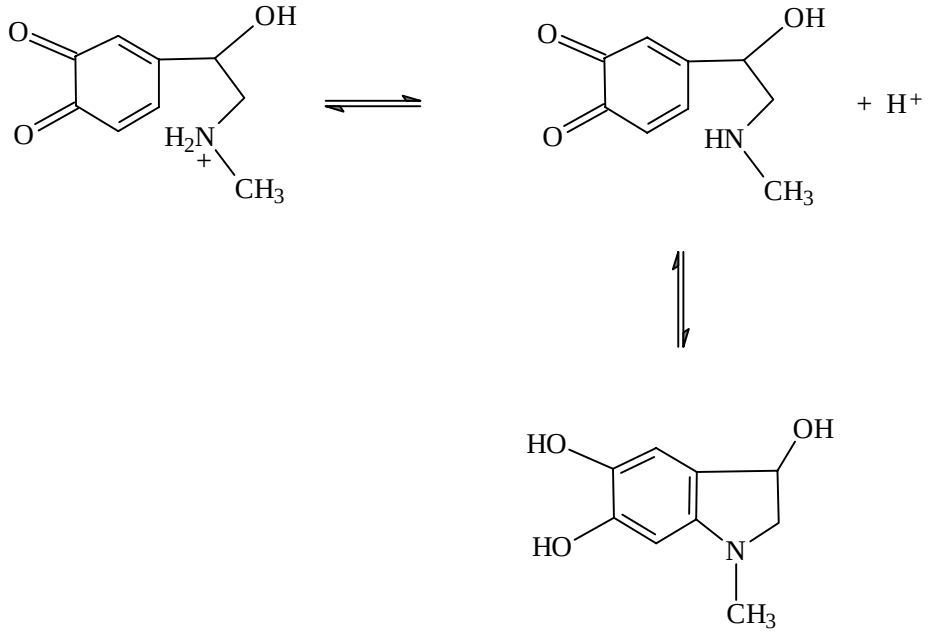
Electrochemistry of Catecholamines

Adrenaline (also called epinephrine) is a hormone of the class of compounds known as catecholamines. Its electrochemical and chemical reaction mechanisms have been previously elucidated [5]. This neurotransmitter undergoes a quasi-reversible two-electron oxidation as follows:



Adrenaline Adrenalinequinone

Under extremely acidic conditions, only the oxidation of adrenaline to adrenalinequinone is observed. However, sufficient unprotonated quinone is present at or above pH 3 to allow for the cyclization reaction to occur according to the following scheme:



Leucoadrenochrome

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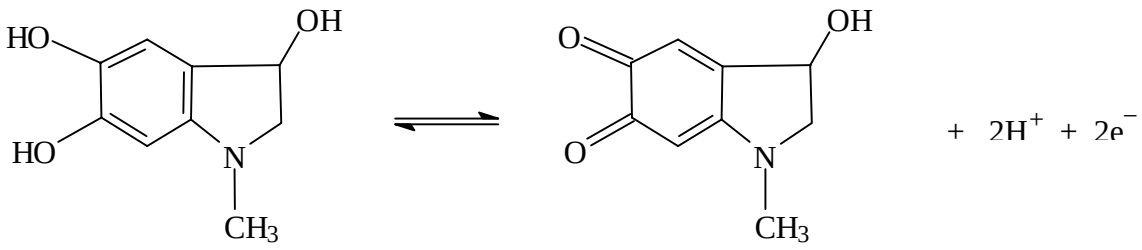
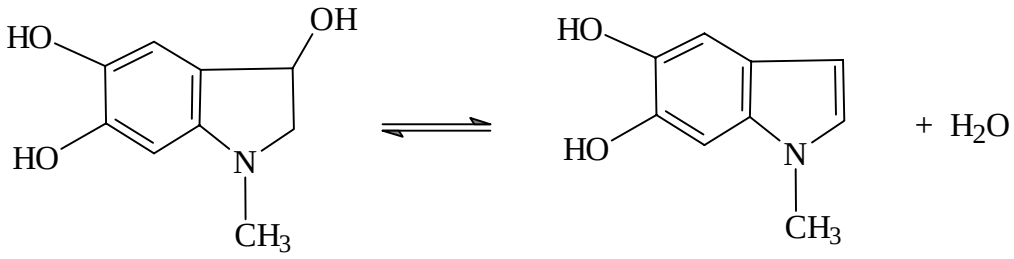
This cyclized product, leucoadrenochrome, is further oxidized to form adrenochrome:



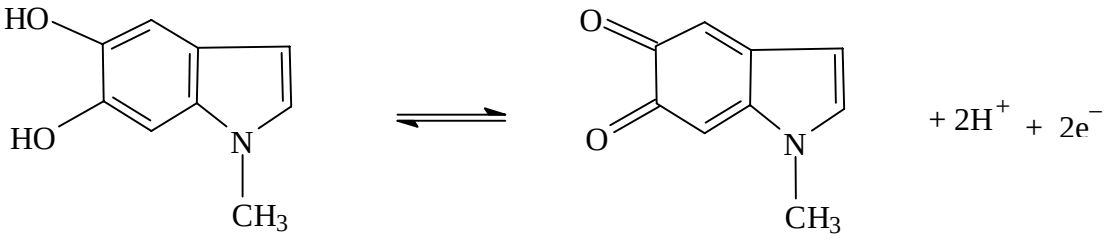
Adrenochrome

Leucoadrenochrome is also dehydrated to 5,6-dihydroxy-*N*-methylindole:



5,6-Dihydroxy-*N*-methylindole

This dehydrated product also undergoes further oxidation:



N-Methylindole-5,6-dione

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As stated above, the identification of each species that gives rise to a voltammetric wave has been established through the electrochemical characterization of pure intermediates [5]. The overall mechanism for the process described above is summarized in Scheme 1.

Scheme 1

$$\begin{array}{lcccccl} A & + & 2\,H^+ & + & 2\,e^- & \rightleftharpoons & B \\ A & \rightleftharpoons & C & + & H^+ & & \\ & & \downarrow & & & & \\ & & D & \rightarrow & F & & \end{array}$$

A = adrenalinequinone, B = adrenaline, C = deprotonated adrenalinequinone, D = leucoadrenochrome, and F = 5,6-dihydroxy-*N*-methylindole. Both F and D each further undergo a two-electron oxidation. Note that by initially scanning from a negative to positive potential, the first reaction in this scheme is driven as follows: $B \rightarrow A + 2\,H^+ + 2\,e^-$.

Experimental

Materials

Solutions of 0.10 M sodium acetate and 0.10 M acetic acid were prepared, and combined in appropriate amounts in a 500 mL beaker to make approximately 200 mL solutions of pH 4 and pH 6. A ca. 2 mM solution (50.00 mL) of adrenaline (Sigma, St. Louis, MO, E-1635) was prepared, and 10.0 mL was pipetted into each of three 50.00-mL volumetric flasks. Each volumetric flask was filled with one of the following: a) 1.2 M H_2SO_4, b) pH 4 acetate buffer, and c) pH 6 acetate buffer.

Electrochemistry

An electrochemical cell was constructed of a 100-mL beaker and a machined Teflon cover. The cover contained openings for three electrodes, as well as gas inlet and outlet ports. Enough solution (approximately 50 mL) was placed in the electrochemical cell to immerse a fresh carbon-paste working (indicator) electrode (3-mm diameter, BAS, Lafayette, IN), a platinum counter (auxiliary) electrode, and saturated Ag/AgCl reference electrode ($E^{\circ\prime}$ = 199 mV). The solution was deaerated by bubbling nitrogen through the solution for 5 minutes, and then by continually flowing nitrogen gently

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over the surface of the solution. An alternative microscale (25-μl) electrochemical cell may also be used [6]. Cyclic voltammograms were obtained using a BAS CV-50W potentiostat.

The shape of the voltammograms was fairly sensitive to electrode preparation. It was important to prepare a fresh carbon electrode for each pH experiment. Carbon paste in Nujol mineral oil (BAS, Lafayette, IN) was gently pressed into the cavity of the working electrode, ensuring that there were no entrapped gas bubbles. Carbon-paste electrodes, which were used in a previous study [5], were found to be quick and easy to prepare, and they yielded better results than other conventional electrodes (e.g., platinum, glassy carbon, pyrolytic graphite). The scan range was from +1200 mV to –400 mV versus saturated Ag/AgCl, with an initial potential of –400 mV, 4 scan segments (note: two scan segments comprise a forward and a reverse scan), and variable scan rates of 10–500 mV/s. Initial and final potentials are usually chosen to be at least 100 mV past the peak cathodic and anodic potentials. All other potentials in this study were reported versus NHE.

Simulations

In the second laboratory session, DigiSim (BAS, Lafayette, IN) was used to simulate cyclic voltammograms based on an electrode reaction represented in Scheme 1. It is recommended that DigiSim be used on at least a 486DX-based PC. Input parameters for the simulations were as follows: grid = 0.5, step potential = 0.009, 2 cycles, initial potential = –200 mV, switching potential +1400 mV, diffusion coefficients (D) = 1×10^{-5} cm^2/s, uncompensated resistance = 200 Ω, double layer capacitance = 5 μF, and pre-equilibrium disabled. The switching potential is the potential at which the scan is reversed. The value of the diffusion coefficient for adrenaline, a parameter that describes its movement with time in solution, was not measured but reflects a value typical of small molecules. The uncompensated solution resistance and double layer capacitance are parameters that influence the shape of the voltammogram through nonfaradaic processes (current resulting for no net exchange of electrons between the solution and the electrode). These later two parameters will be sensitive to cell design and solution composition. Disabling pre-equilibrium means that prior to imposing the initial potential, no equilibrium is established between the electrode and solution.

The input parameters for the scan rate were identical to those used in the experiment (i.e., 10–500 mV/s). Parameters for the electron-transfer and chemical steps are listed

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TABLE 1. Cyclic Voltammetry Simulation Parameters for Adrenaline Oxidation

	Electron-transfer steps		Chemical steps	
	($E°'$)	$k°$	K	k_f
A + 2 e^- + 2 H^+ ⇌ B	0.51	0.0001	2	10
I + 2 e^- + 2 H^+ ⇌ F	0.50	0.04	1	100
G + 2 e^- + 2 H^+ ⇌ D	0.15	0.005	0.4	30
A ⇌ C + H^+			10^4	10^5
C ⇌ D			0.4	500
D ⇌ F			0.4	500

$E°'$ is the formal reduction potential, $k°$ is the heterogeneous rate constant, K is the equilibrium constant, and k_f is the homogenous rate constant for the forward chemical reaction. A = adrenalinequinone, B = adrenaline, C = deprotonated adrenalinequinone, D = leucoadrenochrome, F = 5,6-dihydroxy-*N*-methylindole, G = adrenochrome, and I = *N*-methylindole-5,6-dione.

in Table 1. Due to theoretical considerations, DigiSim treats reaction mechanisms that are greater than second order, such as A + 2 H^+ + 2 e^- ⇌ B, as multiple second order reactions. Thus, a two-electron-transfer process (n = 2) is treated as two individual single-electron-transfer steps (n = 1). That is, the first step is A + e^- ⇌ A^-, followed by an additional electron-transfer step as A^- + e^- ⇌ B, yielding an overall mechanism of A + 2 e^- ⇌ B. Likewise, a proton-linked electron-transfer mechanism is treated in the same manner. For example, the mechanism for a (n = 2) proton-linked electron-transfer mechanism was treated as indicated in Scheme 2, which yields an overall net reaction of A + 2 H^+ + 2 e^- ⇌ B.

Scheme 2

$$A + e^- \rightleftharpoons A^- \quad (E)$$
$$A^- + H^+ \rightleftharpoons AH \quad (C)$$
$$AH + e^- \rightleftharpoons AH^- \quad (E)$$
$$AH^- + H^+ \rightleftharpoons B \quad (C)$$

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The process illustrated in Scheme 2 is referred to as an ECEC mechanism, and represents one of many possible mechanisms for this proton-linked two-electron-transfer reaction (e.g., a CECE mechanism). An ECEC mechanism means that there is an electron-transfer step (E), followed by a chemical step (C), an electron-transfer step (E), and finally a chemical step (C). Parameters used in the simulations for the chemical steps shown in Scheme 2 are discussed below. The values of the equilibrium constants for the chemical steps (K) and for the rate constant for the forward chemical reaction (k_f) were input as listed in Table 1.

Results and Discussion

Cyclic voltammograms of adrenaline at various pH levels are shown in Figures 2 and 3. In 1.2 M sulfuric acid, only a single voltammetric wave is observed for the oxidation of adrenaline (see Figure 2A). At higher pH levels, additional voltammetric waves are observed due to the formation of additional electroactive species generated from coupled chemical reactions (see Figure 3A). These voltammetric waves, as labeled in Figure 3A, have been previously identified through the electrochemical characterization of pure intermediates as follows [5]: Peak 1, oxidation of adrenaline (B → A, see Scheme 1); Peak 2, reduction of adrenalinequinone (A → B); Peak 3, reduction of adrenochrome (G → D, note that species G, adrenochrome, is not shown in Scheme 2); Peak 4, oxidation of leucoadrenochrome (D → G); and Peak 5, oxidation of 5,6-dihydroxy-*N*-methylindole (F → I, note that species I, *N*-methylindole-5,6-dione, is not shown in Scheme 2). These waves were labeled using the same notation as previously described [5] in order to prevent confusion. Students are required to read this original article as a part of this exercise.

Based on their experimental results and Scheme 2, students are required to qualitatively comment on the pH effect. For example, how does pH influence the rates of 1) the cyclization reaction (C → D), 2) the dehydration reaction (D → F), and 3) the leucoadrenochrome–adrenochrome reaction (D → G)? Students are to probe these pH-induced changes in reaction rates through a comparison of voltammograms as a function of scan rate. We intentionally have not included these data here to ensure that students need to do these experiments to confirm their predictions. CV simulations can be used to further test their ideas as suggested below.

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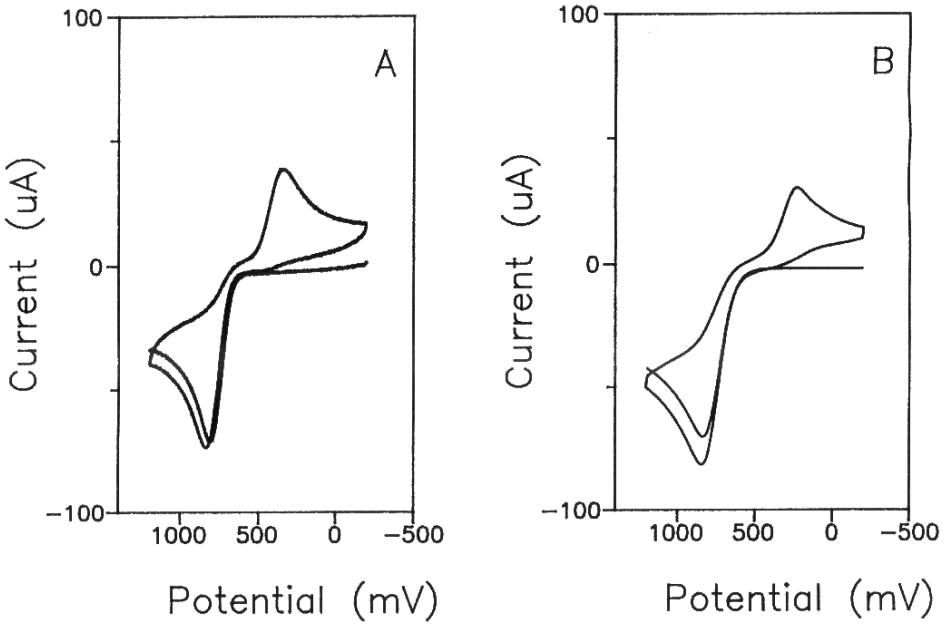

FIGURE 2. EXPERIMENTAL (A) AND SIMULATED (B) CYCLIC VOLTAMMOGRAMS OF 1.5×10^{-4} M ADRENALINE IN 1.2 M H_2SO_4. SCAN RATE IS 400 MV/S.

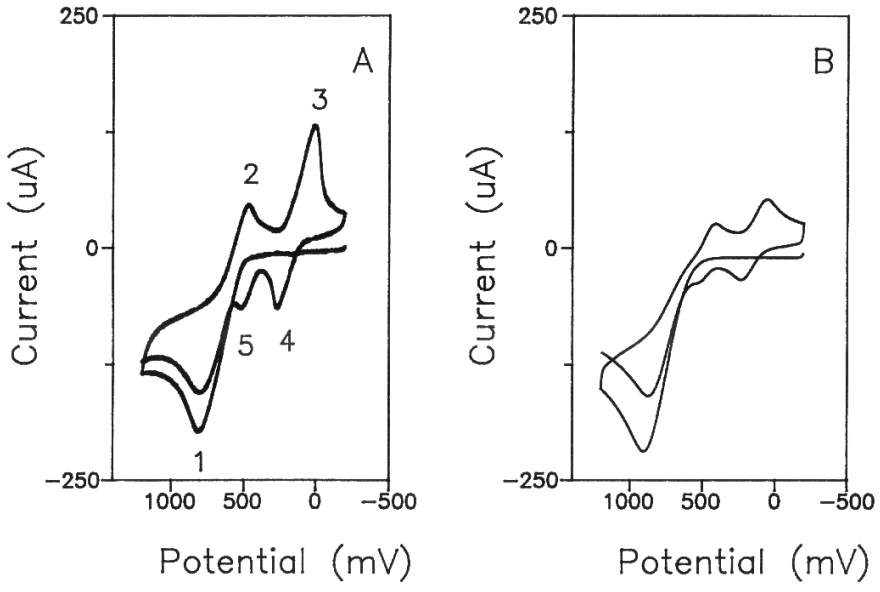

FIGURE 3. EXPERIMENTAL (A) AND SIMULATED (B) CYCLIC VOLTAMMOGRAMS OF 4.2×10^{-4} M ADRENALINE IN pH 4 ACETATE BUFFER. SCAN RATE IS 200 MV/S.

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Simulations

Based on the mechanism and experimental parameters as listed in Scheme 1 and Table 1, respectively, students use DigiSim to reproduce their experimental observations as a function of pH and scan rate. For example, comparisons between experimental and simulated voltammograms of adrenaline are illustrated in Figures 2 and 3. Students are to change the original parameters used in the calculations (e.g., $E^{\circ\prime}$, k°, K, and k_f) as listed in Table 1, and comment on the sensitivity of the calculations to the changes that they made. For example, the students may investigate how the general shape of the voltammogram changes when the rate constant (k_f) for the protonation step of adrenaline is changed. The values of the equilibrium constant (K) and forward rate constant (k_f) for the chemical steps (i.e., protonation) shown in Scheme 2 are not known. The values in Table 1 were empirically derived, and it is recognized that other values may better reproduce experimental results. Nonetheless, it is informative for the students to qualitatively investigate the theoretical change in the shape of the cyclic voltammograms by selecting and changing these values. The suggested values in this table allow for a reasonable reproduction of the experimentally observed changes in the voltammograms of adrenaline as a function of pH. Students are to report other values that were examined, how these different values influenced the shape of the voltammograms, and compare these simulations with their experimental observations for the voltammograms of adrenaline that they recorded at different pH levels.

It is important for students to note that currently there are no inverse algorithms for CV responses which automatically generate a correct mechanism and optimized parameters for a particular electrochemical reaction. For simple mechanisms (e.g. a reversible electron-transfer process), a more quantitative approach for simulating voltammograms is available [7]. Nonetheless, this qualitative simulation of the electrochemical oxidation of adrenaline provides an interesting visual example of a complex electron-transfer mechanism. Moreover, it also illustrates a realistic electrochemical problem—not all electron-transfer systems are simple, rapid and reversible.

CV-Movie

DigiSim also generates concentration profiles as a function of time for the calculated voltammograms. Concentration profiles illustrate the concentration of individual species as a function of distance from the electrode surface. An example of these profiles, referred to as a CV-movie, will accompany this article for a simulated cyclic

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voltammogram of adrenaline if a demonstration version of this program becomes available on the Internet. This movie helps the students visualize the changes in concentration of each species near the electrode surface as a function of time.

Summary

An undergraduate laboratory exercise described above entails the electrochemical characterization of oxidation products of adrenaline as a function of scan rate and pH. The objective of this experiment is to use information obtained through cyclic voltammetry and simulation software to develop a qualitative understanding of the oxidation mechanism of adrenaline.

ACKNOWLEDGMENT

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