CAPSULES
notes and applications from Bioanalytical Systems, Inc.

Mechanistic Study of Acetaminophen Oxidation Using Cyclic Voltammetry - CV-27

Purpose

Cyclic voltammetry (CV) is an invaluable electroanalytical technique widely used in academic research laboratories and industry. Despite this, exposure to electrochemistry in the undergraduate teaching laboratories is usually limited to trace metal analysis, and often ignores this versatile technique altogether. This capsule gives a practical application of cyclic voltammetry for acetaminophen (the active ingredient in Tylenol) suited for the teaching laboratory (1), while at the same time offering an interesting alternative to the more conventional experiments. It demonstrates how CV can be used both quantitatively, to determine an unknown concentration of acetaminophen in a Tylenol tablet, and qualitatively to demonstrate the mechanistic information which can be obtained from CV's.

Acetaminophen (N-acetyl-p-aminophenol, APAP), the active ingredient in Tylenol, is a widely used analgesic. One side effect, though, is that when administered in large doses, liver and kidney damage may result. One of the primary methods of metabolism in the liver involves oxidation of the drug. Therefore, it is advantageous to study the oxidation (redox) chemistry of APAP and its metabolites (2). Thus, this experiment gives a practical application of how CV is used for the determination of mechanistic information.

Experimental

Reagents - Prepare the following supporting electrolyte solutions (1):

(a) Prepare approx. 800 ml (0.5 M) of pH 2.2 McIlvaine buffer by adding 75.3g citric acid (F.W.=210 g/mol) and 11.1g sodium phosphate dibasic (F.W.=268 g/mol) to 800 ml deionized water.

(b) Prepare approx. 200 ml (0.5 M) of pH 6 McIlvaine buffer by adding 4.6g citric acid and 21.0g sodium phosphate dibasic to 200 ml deionized water.

(c) Prepare 200 ml of 1.8 M H_2SO_4 by diluting 20 ml of concentrated (18 M) H_2SO_4 to a total volume of 200 ml using deionized water.

Prepare a 100 ml stock solution of 0.070 M APAP in 0.05M perchloric acid (Note: APAP solution concentrations do not have to be exact but the true concentration should be known accurately). Keep this solution refrigerated when not in use. From this stock solution, prepare 100 ml of 3mM APAP in each of the three supporting electrolyte solutions listed above (only approx. 10 ml of sample is needed for analysis but the larger volumes minimize errors in measurement).

Four additional 100ml solutions should be prepared with concentrations ranging from 0.1 to 5.0 mM (0.1, 1.0, 2.0, and 5.0 mM gave good results). This is done in order to establish a calibration curve for an unknown concentration (see Fig. 3).

An unknown solution can be prepared by dissolving a Tylenol tablet in 250 ml of pH 2.2 buffer, then diluting a 5 ml aliquot of this solution to 50 ml in pH 2.2 buffer (a substantial amount of time is required to dissolve the Tylenol tablet, so it is advantageous to analyze the other samples while waiting).

Setup - Before turning on the CV-27 Voltammogram, check the rear panel and X-Y Recorder to see if the following settings and connections are made (see Fig. 1):

I Polarity = + rdn.
Filter = 0.001
E Polarity = -

For Y-axis:

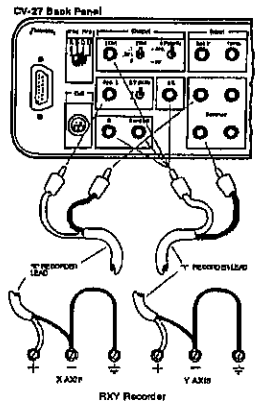
Clear wire- plug in **I OUT** of CV-27 and **H** (red cap) of Y1 of X-Y Recorder

Black wire- plug in **Common** of CV-27, then **L** (black cap) and **G** (blue cap) of **Y1** of X-Y Recorder

For X-axis:

Clear wire- plug in **Appl. E** of CV-27 and **H** (red cap) of X of X-Y Recorder

Black wire- plug in **Common** of CV-27, then **L** (black cap) and **G** (blue cap) of **X** of X-Y Recorder



Assemble Cell - Plug the cell lead cable in the **Cell** socket on the CV-27 rear panel. Add the solution to be analyzed to the cell (i.e., 1mM APAP in pH 2.2 buffer), place the cap on and lower the electrodes into solution. Next, attach the wires to the electrodes (red = auxiliary(Pt wire), black = working(Glassy Carbon), white = reference(Ag/AgCl)). Finally, degass the solution with N_2 for 5-10 minutes in order to eliminate any O_2 interferences.

Set Parameters - On the front panel, set **CELL MODE** to standby **(STBY)**, turn **Power** on, and set **GAIN** = 0.005 mA/V. Turn the **DISPLAY** knob to **App. E.** Next, set **FUNCTION** = **E1**, then **INITIAL E** = 0.00V by turning the **E1** outer (small) knob.

To enter the upper limit of the scan, change the **DISPLAY** to **+ Lim**, then set **E LIMIT** = 1.00V by turning the **+** outer (small) knob.

To enter the lower limit of the scan, change the **DISPLAY** to **- Lim**, then set **E LIMIT** = -0.2 by turning the **-** inner (small) knob.

To set the scan rate, change the **DISPLAY** to **Scan Rate**. Set **SCAN** = 0.1 V/s, then turn the **SCAN RATE** knob until 40 mV/s is on the display.

The parameters of the voltammograph are now set so that the potential is scanned positively from 0.00 V to 1.0 V, at which point the scan direction is reversed, causing a negative scan back to -0.2 V , followed by another reversal and scan back to the original potential of 0.0 V (a cycle is completed; hence, the name " cyclic" voltammetry).

Recorder -

Step 1- Turn the X-Y Recorder **Power** on.

Step 2- Insert graph paper and align with lower left corner.

Step 3- Turn **CHART** toggle to **HOLD**, and **X SERVO** to **ON**.

Step 4- Set **X RANGE** = 50 mV/cm, and **Y-RANGE** = 0.5 V/cm. These settings translate into 50 mV/cm for the **X** or potential(E) axis and 2.5 uA (0.5 V/cm x 0.005 mA/V or **Y- RANGE x GAIN**) for the **Y** or current(i) axis of the voltammogram.

Step 5- Set **PEN** to **DOWN**, then adjust the position of the pen using the **X** and **Y POSITION** controls.

Step 6- When the voltammogram is complete, it can be removed by flipping the **CHART** toggle to **RELEASE** and removing the paper.

Electrode Polishing - Before each scan the glassy carbon working electrode is polished so as to maintain a clean electrode surface. This is done by placing a few drops of alumina on the velvet pad and moistening with distilled water. The electrode surface is then pressed against the alumina on the pad and rotated in a "Figure 8" manner for 1-2 minutes. The electrode tip is then rinsed with distilled water, wiped, and this repeated twice more. Finally, it is rinsed with methanol and allowed to air dry. If a clean surface is not observed, it may be necessary to sonicate for 5 minutes.

Run Voltammogram - If not done already, a 1 mM solution of APAP in pH 2.2 buffer should be added to the cell.

Step 1- Change the **DISPLAY** to App E in order to view the applied potential.

Step 2- Flip the **DIRECTION** toggle to **POS.** in order to ensure that the potential will scan positively.

Step 3- Run the voltammogram by turning **CELL MODE** to **CELL**, then **FUNCTION** to **SCAN.** The voltammogram will sweep through a cycle and continue to cycle until stopped.

Step 4- In order to stop the scan, put **PEN UP** and turn **FUNCTION** to **HOLD.**

Step 5- Finally, set **FUNCTION** back to **E1**, and **CELL MODE** to **STBY** in order to disconnect the cell from the CV-27 electronics.

Step 6- Change graph paper. A cyclic voltammogram as shown in Fig. 2 is obtained using the CV-27 with an XY Recorder.

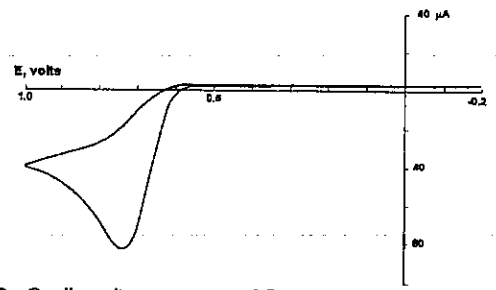


Figure 2. Cyclic voltammogram of 5 mM APAP in pH 2.2 McIlvaine buffer. Glassy carbon electrode. Scan rate = 40 mV/s.

Concentration Effects - To study the effects of varying concentration and to determine an unknown concentration, CV's are obtained for each of the 0.1, 1.0, 2.0, 3.0, 5.0 mM, and unknown APAP solutions in pH 2.2 buffer using a scan rate of 40 mV/s. To do this, repeat the procedure outlined above in "Run Voltammogram" for each solution. Before each scan be sure to polish the working electrode with alumina and degass the solution with N_2 for 5-10 minutes. A plot of current vs. concentration (see Fig. 3) can be done and the unknown concentration determined by extrapolation.

Note: In order to maximize or minimize the size (height) of the plot, adjust the **GAIN** on the CV-27; higher values decrease the size of the plot, lower values increase the size of the plot. If this is not done, the voltammogram may be too large or small for the paper. A "dry" run may be performed with the **PEN UP** to avoid this. For the concentrations 1.0, 2.0, 3.0, and 5.0 mM APAP, the respective settings of 0.005, 0.01, 0.02, and 0.02 mA/V for **GAIN** gave the best results. For the 0.1 mM APAP, set **GAIN** = 0.002 mA/V and **Y-RANGE** = 0.25 V/cm. The Y or current (i) axis values can be determined by multiplying **Y-RANGE** by the gain **(Y-RANGE x GAIN)** to obtain current per cm. The X or potential (E) axis remains unchanged at 50 mV/cm.

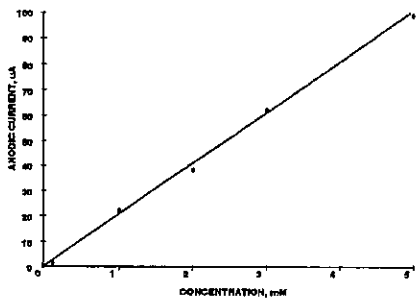


Figure 3. Plot of i_{pa} versus concentration for the 0.1, 1, 2, 3, and 5 mM APAP standards.

Mechanistic Study - In order to gain insight into the mechanism of oxidation of APAP, the 3mM solutions of APAP in pH 2.2 buffer, pH 6 buffer, and 1.8 M H_2SO_4 are analyzed at scan rates of 40 mV/s and 250 mV/s. This can be done by making some simple modifications to the procedures above. Also, it is recommended to run CV's on the buffer solutions alone in order to obtain a background or "blank" voltammogram.

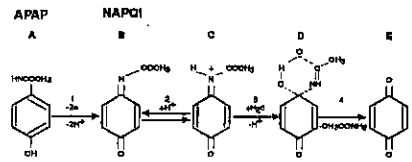
The 3 mM APAP in pH 2.2 buffer can be analyzed at a scan rate of 40 mV/s by repeating the procedure outlined above in "Run Voltammogram". A CV can be obtained at a scan rate of 250 mV/s as follows: change the **DISPLAY** to **Scan Rate**, set **SCAN** = 1 V/s, then turn the **SCAN RATE** knob until 0.250 V/s is on the display. Change the **DISPLAY** back to **App E** and follow the procedure outlined above in "Run Voltammogram".

The 3mM APAP solutions in pH 6 buffer, and in 1.8 M H_2SO_4 can be analyzed in a similar manner. However, for the 1.8 M H_2SO_4 sample , allow the CV to scan three sweep segments, instead of two as in the previous CV's, (i.e., 0.0 V to +1.0 V, then +1.0 V to -0.2 V, then back to +1.0 V) in order to observe the anodic wave corresponding to the oxidation of hydroquinone, the reduction product of benzoquinone. Perform this using both the 40 and 250 mV/s scan rates.

Note: **GAIN** settings of 0.02 mA/V and 0.05 mA/V (**Y-RANGE** = 0.5 V/cm) for the 40 and 250 mV/s scan rates, respectively, were found to give the best results.

Discussion

The oxidation mechanism of APAP was determined to be:



The initial reaction of APAP is an electrochemical oxidation by a two-electron, two-proton process to generate NAPQI (step 1). All subsequent reactions are nonelectrochemical but pH dependent processes. Therefore, by varying solution pH and scan rate of the experiment, information can be extracted and pieced together in order to elucidate a mechanism.

At pH 6 or greater, the solution is not sufficiently acidic to protonate NAPQI. Therefore, no subsequent chemical reactions are observed after the initial electrochemical oxidation of APAP (step 1). CV's for APAP at pH 6 support this (see Figs. 4A,4B). Single, reasonably well defined cathodic and anodic peaks indicate that only two electroactive species are involved. The anodic wave represents the oxidation of APAP to NAPQI, while the cathodic wave represents the reverse reaction (NAPQI to APAP). The similar appearance of the 40 and 250 mV/s scans indicates that both species are stable in the time domain of the CV experiment (scan).

At lower pH's such as 2, the solution is sufficiently acidic to protonate NAPQI (step 2), giving a relatively unstable, but electrochemically active intermediate (C). This intermediate rapidly undergoes hydration to form an electrochemically inactive species (D). CV's of APAP at pH 2.2 are consistent with this

(see Figs. 5A,5B). The anodic current represents the oxidation of APAP. The absence of a well-defined cathodic current, as in Figs. 5A and 5B, indicates that any NAPQI generated by the oxidation rapidly undergoes a chemical reaction to form an electrochemically inactive species (D). At higher scan rates, the rate of reaction is sufficiently slow that the voltammetry can detect some of the electroactive intermediate (C), as shown by the poorly defined cathodic wave in Fig. 5B.

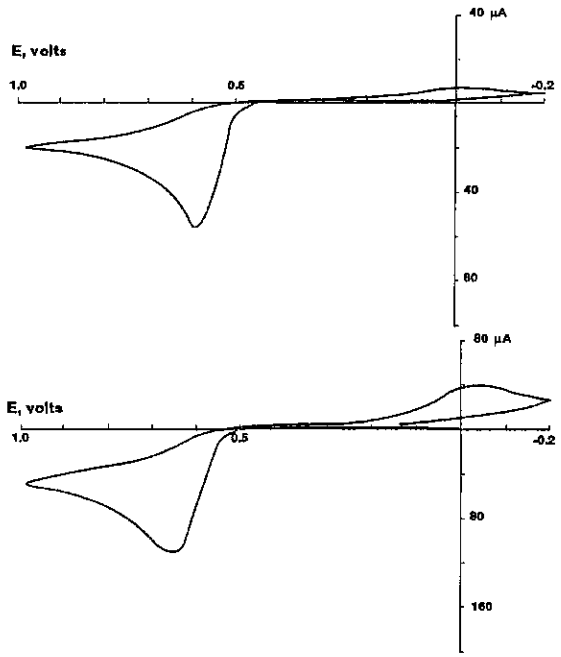


Figure 4. Cyclic voltammograms of 3 mM APAP in pH 6 McIlvaine buffer. Glassy carbon electrode. (A) Scan rate = 40 mV/s. (B)Scan rate = 250 mV/s.

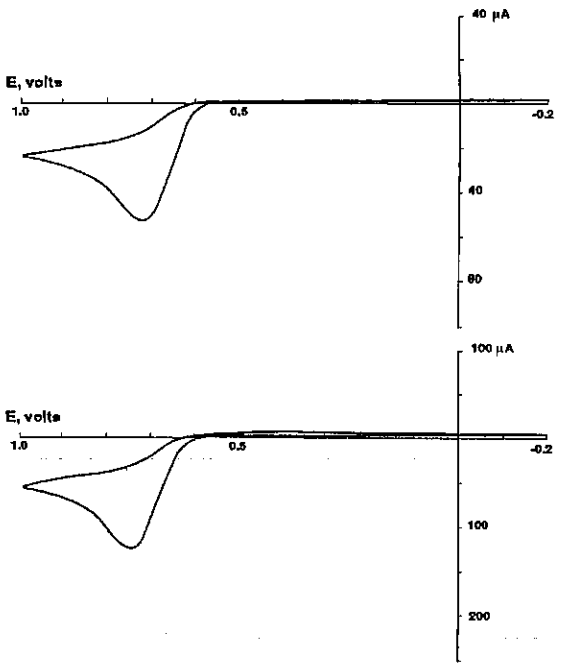


Figure 5. Cyclic voltammograms of 3 mM APAP in pH 2.2 McIlvaine buffer. Glassy carbon electrode. (A) Scan rate = 40 mV/s. (B) Scan Rate = 250 mV/s.

At longer periods of time, hydrated NAPQI (D) converts to benzoquinone (E). However, the solution must be extremely acidic in order to see the formation and subsequent reduction of benzoquinone in the time frame of the CV experiment. Thus, for the CV's obtained in 1.8 M H_2SO_4 (see Figs. 6A,6B), the anodic wave corresponds to the oxidation of APAP to NAPQI, which in highly acidic media, forms benzoquinone (E), giving the observed cathodic wave. Note that the slower scan rate (40 mV/s) gives a more well-defined reduction (cathodic wave), because it allows more time for the formation and accumulation of benzoquinone. The second scan in the positive direction yields an additional anodic peak corresponding to the oxidation of hydroquinone, the reduction product of benzoquinone.

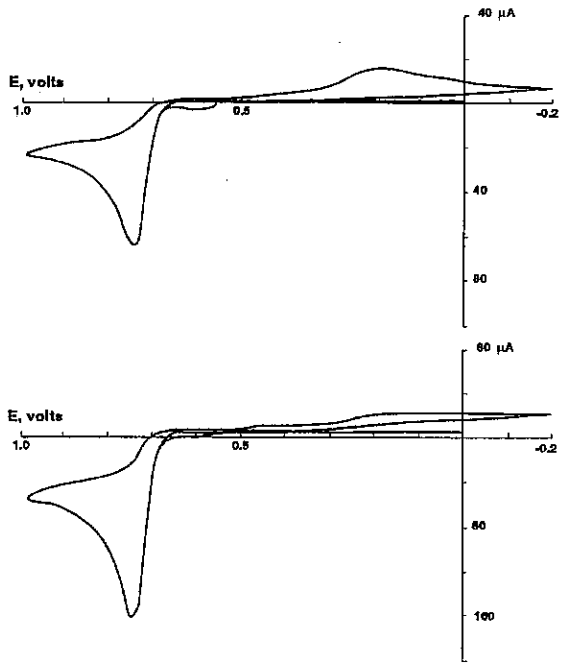


Figure 6. Cyclic voltammograms of 3 mM APAP in 1.8 M H_2SO_4. Glassy carbon electrode. (A) Scan rate = 40 mV/s. (B) Scan rate = 250 mV/s.

(1) Kissinger, P.T., Roston, D.A., Van Benschoten, J.J., Lewis, J.Y., Heinemen, W.R., J. CHEM. ED., 60, 772 (1983).

(2) Miner, D.J., Rice, J.R., Riggin, R.M., Kissinger, P.T., Anal. Chem., 53, 2258 (1981).