CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Mechanistic Study of Acetaminophen Oxidation Using Cyclic Voltammetry - BAS 100B

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.

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 in pH 2.2 buffer 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. 1). 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).

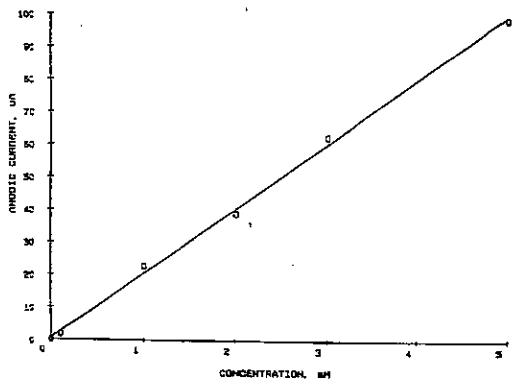


Figure 1. Plot of i_{pa} versus concentration for the 0.1, 1, 2, 3, and 5 mM APAP standards.

Equipment - Assemble the cell by placing the glassy carbon working electrode, platinum wire auxiliary electrode, and Ag/AgCl reference electrode in the solution to be analyzed. Attach the cell lead wire (clips) to the electrodes (red = auxiliary, black = working, white = reference). Finally, degass the solution with N_2 for 5-10 minutes in order to eliminate any O_2 interference.

Procedures

Concentration Study - In order to study the effects of varying concentration, and in order to establish a calibration curve, CV's are obtained for the five standard solutions in pH 2.2 buffer prepared above. Scans are initiated in the positive direction at 0.0 V,

with scan limits set at 1.0 V and -0.2 V and a scan rate of 40 mV/s.

1. A cyclic voltammogram as shown in Fig. 2 can be obtained easily using the BAS 100B Electrochemical Analyzer by using a few simple commands as outlined below.

Step	*Prompt*	*Typed Response*
1	[POWER ON]	↵
2	DATE=##-##-##	↵
3	TIME=##-##-##	↵
4	OPERATING MODE=	CV↵
5	INIT E(MV)=#	0.0↵
6	HIGH E(MV)=	1000↵
7	LOW E (MV)=	-200↵
8	V(MV/SEC)=	40↵
9	SWEEP SEGMENTS=	2↵
10	SENSITIVITY (A/V)=1.0E-#	5↵
11	*	LABEL↵
12	LABEL:	(Type in title)↵
13	* RUN IN PROGRESS	R↵
14	* (Data is scaled to screen)	B↵
15	* (Data is sent to plotter) *	PB↵

2. The subsequent solutions and unknown solution can be analyzed by changing the solution and repeating steps 11-15 after the star prompt (*) appears. Be sure to polish the glassy carbon working electrode, degass, and change the plotter paper after each run. A plot of current vs. concentration (see Fig. 1) can be done and the unknown concentration determined by comparing with the calibration plot.

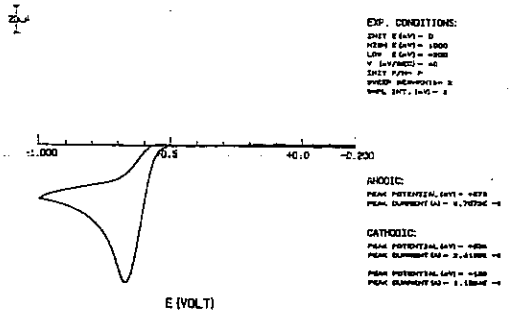
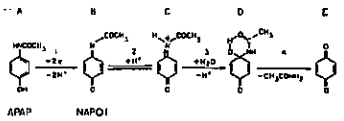


Figure 2. Cyclic voltammogram of 5mM APAP in pH 2.2 McIlvaine buffer. Glassy carbon electrode. Scan rate = 40 mV/s.

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 procedure above. Obtain a CV of the 3mM APAP in pH 2.2 buffer at 40 mV/s scan rate by repeating steps 11-15 of the above procedure. After the star prompt appears, type CG. Proceed from step 5 to step 8 to change the scan rate [V(MV/SEC)=#] to 250 (mV/s). Continue with steps 9-15 to generate a voltammogram. Repeat this for the 3mM APAP in pH 6 buffer solution. However, for the 1.8 M H_2SO_4 sample, set the SWEEP SEGMENTS = 3 in order to observe the anodic wave corresponding to the oxidation of hydroquinone, the reduction product of benzoquinone. This can be done by typing CG after the star prompt. Proceed from step 5 to step 9 to change the sweep segments [SWEEP SEGMENTS = #] to 3. Continue with steps 10-15 to generate a voltammogram. Perform this using both the 40 and 250 mV/s scan rates as above.

Discussion

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. 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 non- electrochemical 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. 3A,3B). 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).

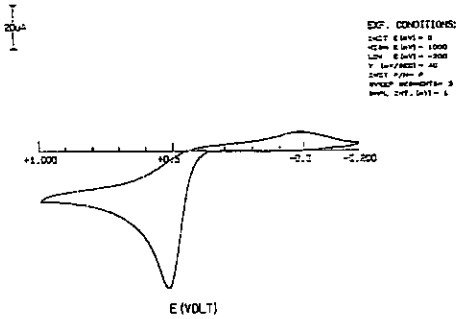


Figure 3(A).Cyclic voltammograms of 3 mM APAP in pH 6 McIlvaine buffer. Glassy carbon electrode. Scan rate = 40 mV/s.

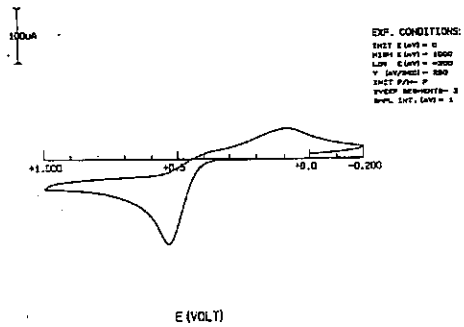


Figure 3(B).Cyclic voltammograms of 3 mM APAP in pH 6 McIlvaine buffer. Glassy carbon electrode. Scan rate = 250 mV/s.

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. 4A,4B). The anodic current represents the oxidation of APAP. The absence of a well-defined cathodic current, as in Figs. 1A and 1B, 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 slow enough to trap or detect some of the electroactive intermediate (C), as shown by the poorly defined cathodic wave in Fig. 4B.

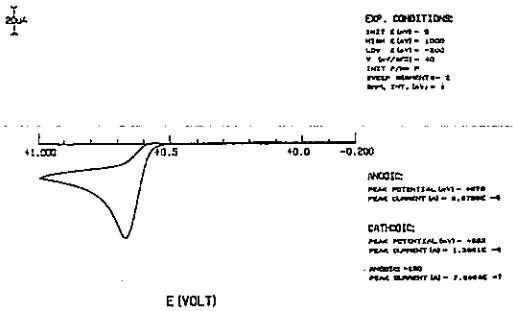


Figure 4(A). Cyclic voltammograms of 3mM APAP in pH 2.2 McIlvaine buffer. Glassy carbon electrode. Scan rate = 40 mV/s.

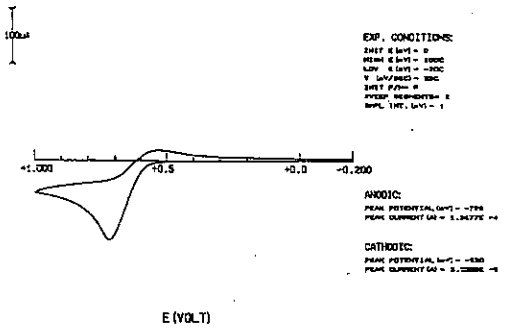


Figure 4(B). Cyclic voltammograms of 3mM APAP in pH 2.2 McIlvaine buffer. Glassy carbon electrode. Scan rate = 250 mV/s.

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. 5A,5B), 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.

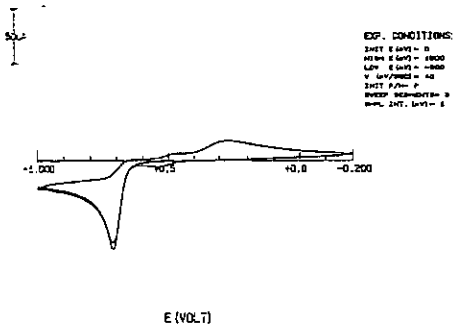


Figure 5(A) Cyclic voltammograms of 3mM APAP in 1.8 M H_2SO_4. Glassy carbon electrode. Scan rate = 40 mV/s.

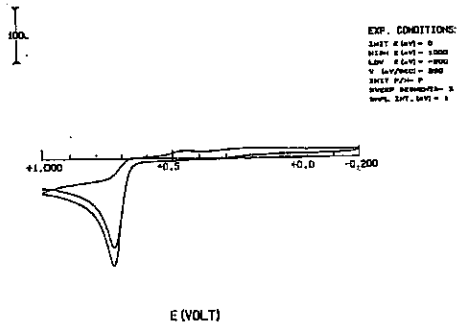


Figure 5(B). Cyclic voltammograms of 3mM APAP in 1.8 M H_2SO_4. Glassy carbon electrode. Scan rate = 250 mV/s.

(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).