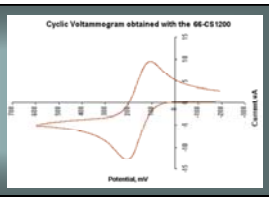
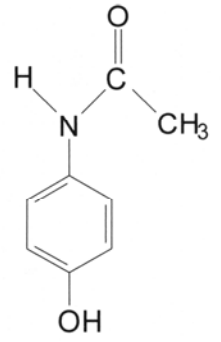


6. Acetaminophen (Tylenol): Electroanalytical Study of Acetaminophen (APAP) by Cyclic Voltammetry

PURPOSE: To determine the oxidative mechanism and the concentration of APAP

BACKGROUND: Acetaminophen (N-acetyl-p-aminophenol) is the active ingredient of Tylenol, a non-opiate, non-salicylate analgesic and antipyretic that occurs as a white, odorless, crystalline powder with a molecular formula of $C_8H_9NO_2$. Its formula weight is 151.16 [ref. 1].

Often recommended as a substitute for aspirin (acetylsalicylic acid), it provides temporary relief of minor aches and pain associated with heartburn, acid indigestion, flu and colds. Acetaminophen acts by elevating the pain threshold and antipyresis through action on the hypothalamic heat regulation center. Readily absorbed and distributed throughout fluids in the body, unchanged acetaminophen is excreted in the urine. Products metabolized through the liver appear in the urine within 24 hours [ref. 2]. Unlike opioid analgesics, APAP does not cause euphoria or alter moods in any way, and is completely free of addiction and withdrawal. It went on sale in 1955 as Tylenol. A historical background and discussion of its pharmacology, dosage levels and circumstances of toxicity can be found in http://en.wikipedia.org/wiki/acetaminophen [ref. 3].



acetaminophen

APAP, like many other organic substances, undergoes an electrochemical "*ec*" mechanism. That is, the electron transfer step "*e*" produces a species that undergoes a follow-up chemical reaction "*c*" to produce either an electrochemically active or inactive product. Cyclic voltammetry is an ideal electrochemical method to probe this mechanism since the fate of the species produced in the forward scan can be ascertained during the reverse scan. The scan rate determines the time window for observation. The proposed oxidative mechanism [ref. 4] of APAP is shown in Figure 1.

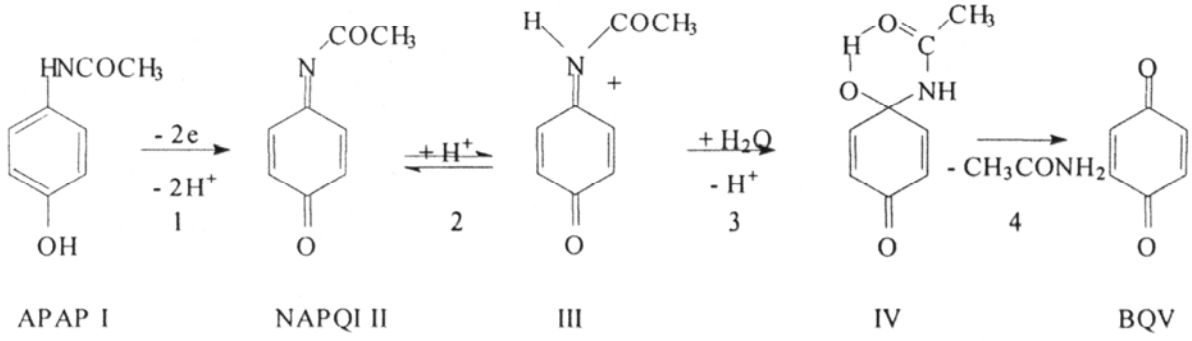


Figure 1. Proposed *ec* mechanism for the oxidation of APAP

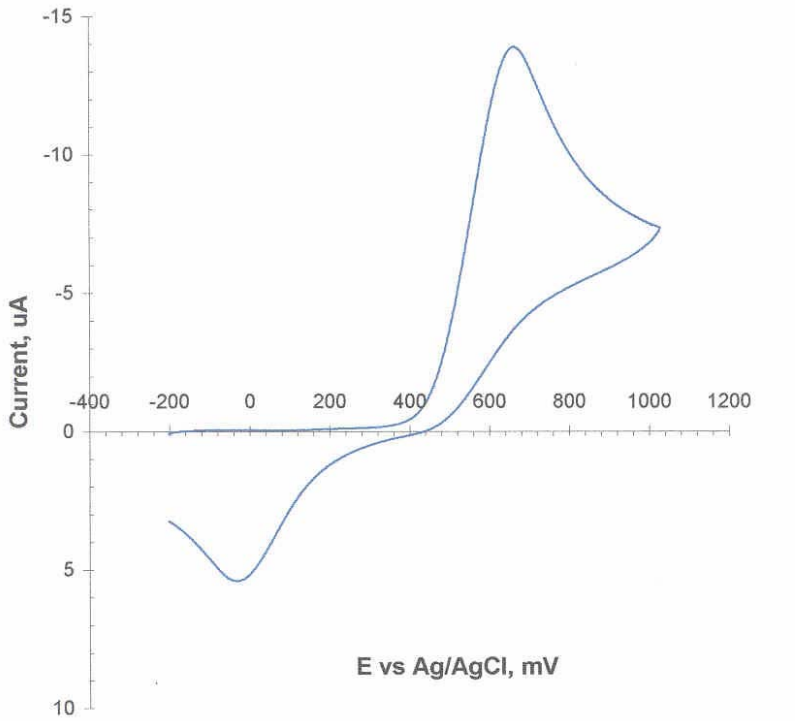


Figure 2. Cyclic Voltammogram of 2 mM APAP in pH 7.0 buffer at a glassy carbon electrode. Scan rate is 100 mV/s.

The initial step is the oxidation of APAP in a pH dependent, two-electron, two-proton step to produce N-acetyl-p-quinoneimine II (NAPQI). A typical cyclic voltammogram (CV) for APAP at pH 7 is seen in Figure 2. The experiment described herein is similar to an online one [ref. 5] and its embedded referrals to the original work by Kissinger and Heineman [6].

The anodic and cathodic waves are both well defined – the cathodic wave being shifted toward negative potentials due to a slower heterogeneous electron transfer rate of the quinoneimine. In acidic solutions, the follow-up equilibrium favors the protonated species III that undergoes rearrangement to the electroinactive species IV. Thus, the reverse cathodic wave is not observed, as illustrated in Figure 3, for CV of APAP at pH 2. If the scan rate is very fast, you may be able to "capture" species II, before it completely converts to species III, and observe a wave on the reverse scan.

The effect of solution pH and scan rate to cyclic voltammograms, and the determination of APAP in a Tylenol sample are the objectives of this experiment. An optional experiment, as described at the end of this section, is the calculation of the rate for the conversion of species III/IV to V, the benzoquinone.

The background to the basics of cyclic voltammetry is discussed in Concepts as linked from the main menu and in Experiment 1, titled *Cyclic Voltammetry at Solid Electrodes.*

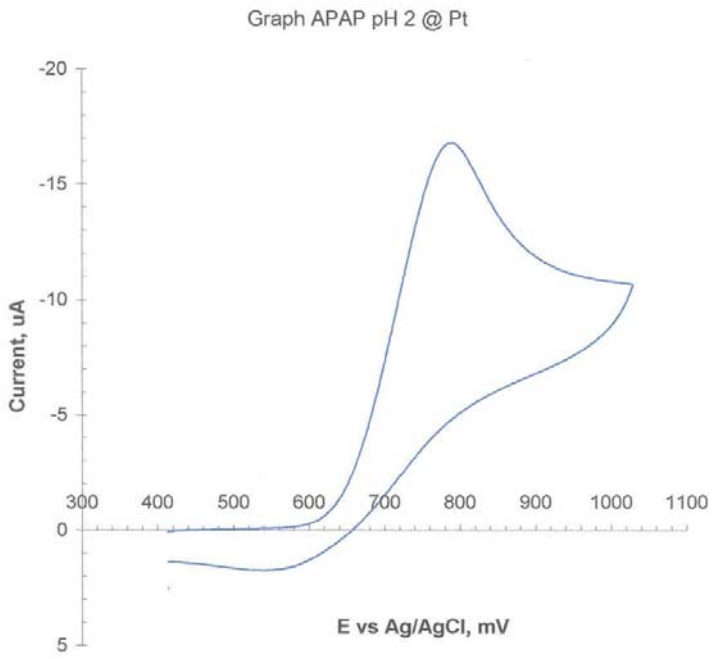


Figure 3. Cyclic Voltammogram of APAP in pH 2 buffer at a Pt electrode. Scan rate is 100 mV/s.

EXPERIMENT

Equipment

- An analog or computer-controlled potentiostat with appropriate data acquisition capability (please see laboratory instructor for directions on operation of potentiostat).
- Electrochemical cell and electrodes
  - Small volume cell
  - 1 – 3 mm glassy carbon or Pt flat tipped working electrode
  - Pt auxiliary electrode
  - Ag/AgCl reference electrode
  - Electrode polishing kit or the electrode maintenance kit

Chemical Solutions

A. Prepare 100 ml stock phosphate buffer solution at pH 7 containing 1 M KCl.
pH 7 (0.25 M Na_2HPO_4 and 0.25 M NaH_2PO_4)

B. Prepare 100 ml stock phosphate buffer solutions at pH 2 containing 1 M KCl.
pH 2 (1 M NaH_2PO_4 and 1 M H_3PO_4)

C. Prepare100 ml stock solution of 0.1 M APAP in 1 mM $HClO_4$ or HCl (calculate the exact concentration of the APAP to 3 significant figures from the amount of APAP weighed)

Procedure

1. It is important to clean the electrode surface prior to each run that involves a change in the solution. Polish the electrode (Pt or GC) with 1 micron or smaller particles of alumina on a clean flat surface, preferably glass. Use of light pressure on the electrode while tracing a figure 8 for 8-10 times is suggested. Rinse the electrode with pure water and sonicate (if available) for 1 minute in a beaker containing pure water. Wipe the edges of the electrode (never the electrodes surface) with clean tissue paper, like Kimwipe™ and use immediately. Further discussion on cleaning and polishing GC is discussed in the Tech Note titled "The Care and Feeding of Electrodes."

2. Prepare 100 ml of 2 mM APAP in pH 7 buffer (pipette 5.0 ml of solution A and 5.0 ml of solution C into a 100 ml volumetric flask and fill to mark with pure water).

3. Insert the working electrode (glassy carbon or Pt), the reference and the auxiliary electrode in the cell and fill the cell with 2 mM APAP solution until the electrodes are covered. Attach electrodes to the potentiostat cable.

4. Set scan rate at 100 mV/s and scan potential range of E_i (initial value) at -200 mV, Es (switching potential) at 1000 mV, and E_f (final) at –200 mV (see Figure 2).

5. See laboratory instructor for set-up and operation direction for either analog or computerized potentiostat.

6. Run duplicate scans, allowing 2-3 minutes between each scan to allow equilibration of the concentrations at the electrode surface with the bulk solution. Repeat at scan rates of 50, 200 and 500 mV/s. You may need to adjust the sensitivity range to see a reasonable current peak on the CV. [Note: the peak current, I_p, varies as a square root of the scan rate].

7. Run duplicate CVs of 2.0 mM APAP in pH 2 phosphate buffer at scan rates of 50, 100, 200 and 500 mV/s.

8. To determine the amount of APAP in your Tylenol sample, run duplicate CVs of APAP at the following concentrations: 10 mM, 5.0 mM, 2.0 mM, 1.0 mM and 0.2 mM at pH 2 with GC or Pt working electrode. Set the scan rate at 100 mV/s and the scan range of 400 mV to 1000 mV. The results will be used to make a calibration plot. Direction for preparing the standard solutions for calibration is as follows -

	Solution B	Solution C	Concentration of standard solution, 50 ml final volume
#1	5.0 ml	1.0 ml	2.0 mM
#2	5.0	2.5	5.0
#3	5.0	5.0	10
#4	5.0	5.0 ml of #3	1.0
#5	5.0	5.0 ml of #1	0.20

9. Obtain a sample Tylenol tablet from the lab supervisor, grind it into a powder with a mortar and pestle, and place 0.03 to 0.05 g of the powder into a 50 ml volumetric flask. Add 5.0 ml of solution B and dilute to mark with pure water. The tablet may contain some insoluble material that does not dissolve. Shake for 5 minutes to assure that the soluble portions dissolve. Run triplicate CVs of the Tylenol.

   Save all CV data on the hard drive or other storage medium with file names for future reference. If data are saved as an ASCII file, you can import data files to Microsoft EXCEL to have flexibility to select different formats for CV graphs.

REPORT (Data Analysis and Discussion)

Consult with the lab instructor about content and format of the report. Suggested content includes graphs, plots, calculations and discussion. Make a suggested "do" list with things to consider and questions to answer [see reference 5 about CV calculations].

1. Show plots of I_p vs. APAP concentration at a scan rate of 100 mV/s, and from the plot calculate and report the concentration of the unknown APAP. Correct the I_p for background charging current by extrapolating the initial straight part at the foot of the wave. With the computer-controlled potentiostat (CS-1200), I_p and E_p values can be determined with the imbedded "math" software, including baseline corrections.

2. Plot I_p vs. square root of the scan rate for the oxidation of APAP from results obtained at pH 2 and 7.

3. Are the plots linear? If not, why not?

4. Does the E_p value for the oxidation of APAP shift with pH? What is the theoretical value of the E_p shift? Show your reasoning.

5. At pH 7, why is the reverse cathodic peak height less than that for the oxidation of APAP?

6. At pH 7, what is the formal potential for step 1?

7. At pH 2, what are the differences in the CVs at slow and fast scan rates? Explain.

8. Are your results consistent with the proposed mechanism in Figure 1?

9. Did the results vary with the way in which you pretreated the electrode? What method of pretreatment produced the lowest E_{pa} value and best reproducibility?

10. Comment on what worked and what did not work for you in this experiment. What changes would you propose to improve the experiment?

OPTIONAL EXPERIMENT:

In strong acid (e.g., 1 to 2 M sulfuric acid) the rate of step 4 becomes sufficiently fast that the reduction of benzoquinone, species V, can be observed on the reverse cathodic scan. If you continue to run successive CV scans, the build-up of benzoquinone and its reduced form, hydroquinone, can be observed by the appearance of a wave just prior to the wave for APAP. The scan rate provides the "time window" for observation of different species in the mechanism.

Run CV of 2.0 mM APAP in 1 M sulfuric acid at scan rate of 20, 50, 100 and 200 mV/s. The potential range for the scan is -200 mV to 1000 mV, and then back to –200 mv. Run 10 continuous CV cycles at a scan rate of 100 mV/s to observe the appearance of waves due to benzoquinone/hydroquinone couple.

The kinetic rate for step 4 → 5, the formation of benzoquinone, is determined by using the method described in reference 7. Discuss the method and results of your kinetic analysis in your Report.

REFERENCES

1. http://www.rxlist.com/cgi/generic/apap_ids.htm

2. http://www.medicinenet.com/acetaminophen/article.htm

3. http://en.wikipedia.org/wiki/acetaminophen

4. D. J. Miner, et al., *Voltammetry of acetaminophen and its metabolites,* Anal.Chem, 53 (1981) 2258.

5. *Study of Electrode Mechanism by Cyclic Voltammetry*, D. Howard Fairbrother: www.jhu.edu/%7Echem/fairbr/OLDS/EPrelab.pdf

6. Peter T. Kissinger and Wm. R. Heineman, J. Chem. Ed., 90 (1983) 702 and 722. A Copy of the *Cyclic Voltammetry* article is online at www.jhu.edu/%7Echem/fairbr/OLDS/CV_Article.pdf.

7. See experiment in this manual titled, *Cyclic Voltammetry of Dopamine: An "ec" Mechanism.*