Technical Note
2008-01

REV 001 (MAR 2008)

Determination of Acetaminophen in a Children's Pain Relief Elixir

An Inexpensive Experiment Using Disposable Screen-Printed Carbon Electrodes

This experiment demonstrates the use of modern electroanalytical chemistry to determine the active ingredient in a popular children's pain relief elixir. Designed for use in an instructional laboratory setting, this procedure describes the use of cyclic voltammetry to verify the concentration of acetaminophen (4-acetamidophenol) indicated on the label for the elixir. Advantages of the approach used here include the use of inexpensive and disposable screen-printed carbon electrodes and the use of an inexpensive consumer-grade supporting electrolyte solution that is widely available on the mass market. The only reagent grade chemical required for this procedure is a small amount of pure 4-acetamidophenol used for standardization.

Introduction

Acetaminophen (4-acetamidophenol) is the active ingredient in several over-the-counter pain relief medications, including the well-known brand, Tylenol®. Acetaminophen has largely replaced aspirin (acetylsalicylic acid) as the medication of choice for children and infants because asprin has, in some cases, been linked to the development of Reye's Syndrome.

For easier dosing of small children, acetaminophen is often formulated as a liquid elixir (see Figure 1), which is a viscous aqueous solution containing various colorings and flavorings. A typical sample of elixir has an acetaminophen concentration in the range from 30 to 100 g/L. In this lab experiment, an electrochemical method known as cyclic voltammetry (CV) is used to determine the acetaminophen concentration in a sample of elixir, and the result is compared with the stated value provided by the manufacturer on the elixir package.

Theory

Acetaminophen is an electroactive molecule which exhibits irreversible electrochemical behavior. When oxidized at an electrode, acetaminophen **(A)** is converted to its quinone form **(B)**, which is called *N*-acetyl-4-quinoneimine and abbreviated NAPQI. This oxidation is a two-electron, two-proton electron transfer process (see Figure 2). In the presence of an acid catalyst, NAPQI is rapidly converted to a hydrate **(C)** called N-acetyl-4-quinoneimine hydrate.

Figure 1: Experimental Apparatus & Chemicals

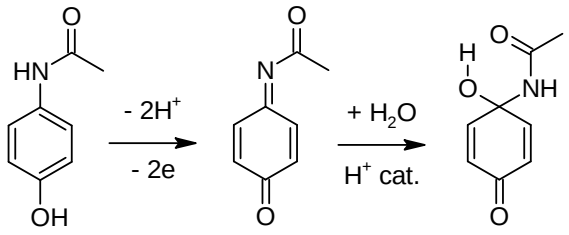


Figure 2: Oxidation of 4-Acetamidophenol

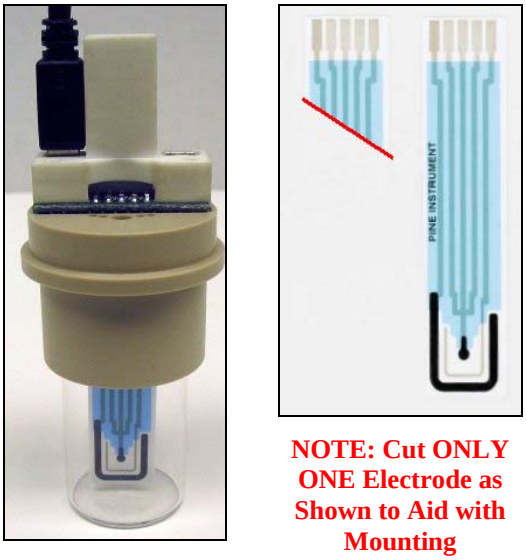
The two-electron oxidation of 4-acetamidophenol **(A)** is observed as an anodic (oxidizing) current when the electrode potential is swept in the positive direction (see Figure 7). In an acidic solution (pH ~ 2), the NAPQI product **(B)** is quickly converted into the hydrate **(C)**. Because the hydrate is electro-chemically inactive, it is not possible to convert the hydrate back to 4-acetomidophenol by reducing it. That is, it is not possible to reverse the process by sweeping the electrode potential back in the negative direction. In an electrochemical context, this type of reaction at an electrode is said to be *irreversible*.

At higher pH, however, the NAPQI intermediate is not as rapidly converted to the hydrate. Unlike the hydrate **(C)**, the NAPQI intermediate **(B)** is, in fact, electroactive. This means that the NAPQI can be converted back to 4-acetamidophenol **(A)** by reversing the process at the electrode. This reversal is accomplished by sweeping the electrode potential back to negative potentials at which the NAPQI **(B)** is reduced back to 4-acetamidophenol **(A)**.

With less acid catalyst present at higher pH, the rate of hydration decreases, resulting in a higher percentage of NAPQI molecules remaining intact and in the vicinity of the electrode surface. So as the solution pH increases, it becomes possible to observe a cathodic (reducing) current at the electrode. Thus, as the pH of the solution increases, the electrochemical process changes from being *irreversible* to being *quasi-reversible*.

In this experiment, cyclic voltammetry is used to observe both the anodic (oxidizing) current and the cathodic (reducing) current as the potential of the electrode is swept first to in the positive direction and then back in the negative direction (see Figure 7). Because of its dependence upon the pH of the solution, the cathodic current is not a good choice for quantitative analysis. The anodic peak current, however, can be used as an analytical signal which is proportional to the concentration of the 4-acetamidophenol **(A)** in the solution.

As is typical in most analytical assays, a set of standard solutions of the analyte (4-acetamidophenol) are prepared and analyzed, and then a calibration curve is constructed to show the relationship between the signal (i.e., the anodic current) and the analyte concentration. After establishing this relationship, a dilute solution of the children's pain relief elixir is prepared and analyzed, and the analyte concentration in the elixir is determined.



NOTE: Cut ONLY ONE Electrode as Shown to Aid with Mounting

Figure 3: Student Voltammetry Cell with Disposable Screen-Printed Carbon Electrodes

Apparatus

- Pine WaveNow™ USB Potentiostat with AfterMath™ Data Analysis Software (Pine part number AFTP1)
- Pine Student Voltammetry Cell (Pine part number AKSPEKIT)
- Disposable Screen-Printed Carbon Electrodes (Pine part number RRPE1001C, two per student)
- 1 mL and 10 mL variable volume pipettes
- 100 mL and 250 mL volumetric flasks

Chemicals and Materials

The following chemicals or reagents are needed to prepare stock solutions, and the amounts given are adequate for a class consisting of 20 students/groups.

Contact Saline Buffer (2.5 L)
This inexpensive borate-based buffer solution is normally used for contact lens rinsing and storage and is available in most drug stores. The major ingredients in this buffer (pH ~ 7.3) are sodium chloride, boric acid, and sodium borate.

4-Acetamidophenol (1.0 gram)
This chemical is available in 98% purity from Aldrich Chemical Company (A7302-100G-A). Its molecular mass is 151.2 g/mole.

Children's Tylenol® Elixir (4 fluid ounces, 118 mL)
This elixir can be obtained from most drug stores as a flavored oral suspension. *Students should make a note of the acetaminophen concentration listed on the elixir label and/or packaging.*

Figure 4: Typical Variable Pipettes

Procedure

A. Solution Preparation

Stock solutions are prepared by the instructor, and the amounts described below are adequate for use by a group of 20 students. When not in use, solutions should be capped and stored in a refrigerator.

Acetaminophen Stock Solution (250 mL)
Precisely weigh 385.6 mg of acetaminophen (98%) into a clean and dry 250 mL volumetric flask. Add about 100 mL of the saline buffer, and swirl the flask gently. Once all solid has dissolved, fill the flask "to the line" using the saline buffer. This stock solution has an acetaminophen concentration near 10.0 mM. Calculate the exact concentration to three significant digits and provide this information to the students.

Elixir Stock Solution (100 mL)
Using a dropping pipette, transfer 2.477 g of elixir into a clean and dry 100 mL volumetric flask. After adding about 50 mL of saline buffer, swirl the flask until the elixir is completely mixed with the buffer. Fill the flask "to the line" using the saline buffer. Students are provided with two pieces of important information about this stock solution. First, they are given the exact mass (3 significant figures) of the viscous elixir solution used to make this stock solution. Second, they are given the density of the undiluted elixir (three significant digits) as previously measured by the instructor. A reasonable density determination typically requires ~50 mL of elixir.

Students should also read the Tylenol label, and write down the acetaminophen concentration in their lab notebook for later reference. Note that the elixir label is likely to list the concentration in an unusual way, such as 160 mg per 5 mL. Students should convert this value to molarity units and also to grams per liter.

stock volume (mL)	buffer volume (mL)	approximate concentration
0.00	10.0	0.00 mM
0.20	9.80	0.20
0.40	9.60	0.40
0.60	9.40	0.60
0.80	9.20	0.80
1.00	9.00	1.00

Table 1: Guide for Preparing Standard Solutions

Standard Solution Preparation
Standard solutions are most efficiently prepared using variable pipettes (see Figure 4) and using Table 1 as a guide. Each individual student (or group of students) prepares these solutions by combining the volumes of stock and buffer solution shown in the table to arrive at ~10.0 mL of each stock solution. Students should pipette the stock and buffer solutions directly into a set of clean and dry 20 mL scintillation vials.

Note that the first solution shown in the table contains only the buffer solution. This solution is used as a "blank" solution in order to obtain a background cyclic voltammogram.

The concentrations of standard solutions will have values near those listed the table, however, the student must calculate the actual concentrations using the precise concentration of the acetaminophen stock solution provided by the instructor.

Diluted Elixir Test Solution (10 mL per student)
The acetaminophen concentration in the Elixir Stock Solution prepared by the instructor is too high for optimal measurements using cyclic voltammetry. Using a variable pipette, each student should prepare 10 mL of diluted solution in a clean and dry 20 mL scintillation vial. The student should attempt to dilute the Elixir Stock Solution by a factor of ten. Students should be sure to use the saline buffer as the dilution solvent (rather than water).

B. Background Voltammogram

A simple background voltammogram of the saline buffer is a good way to confirm several important control characteristics: the purity of the solution, the cleanliness of the glassware, and the cleanliness of the electrode surface, all in a single experiment. Any electroactive impurities from the buffer, the glassware, or the electrode surface will show up as unexplained peaks in the background voltammogram.

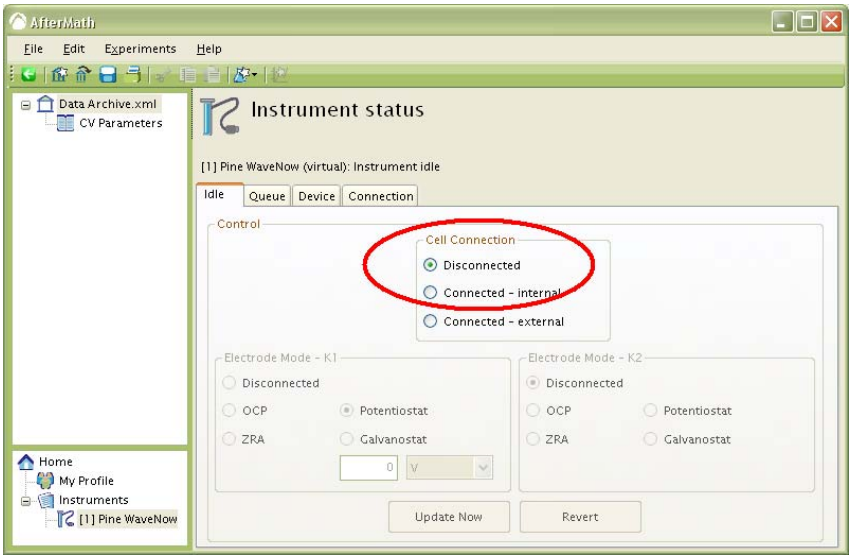


Figure 5: Setting the Potentiostat Idle Condition to Disconnected

1) Turn on the potentiostat and connect it to a personal computer using a USB cable. Use the AfterMath software application to control the potentiostat (see Figure 5). Set the "idle condition" for the potentiostat to "disconnected" as shown.

2) Connect the cell cable to the potentiostat using the end terminated with a HD-15 connector. The other end of the cable should be a mini-USB connector.

3) Obtain two screen-printed electrodes (SPE). These patterned electrodes have a working electrode in the center which has a 2.0 mm diameter. The working electrode area is defined by a blue masking material which coats the underlying carbon ink. Compute the surface area of the working electrode (in cm^2) and make a note of your result in your lab notebook. The electrode pattern also includes a counter electrode (large, black U-shaped electrode) and a silver/silver-chloride reference electrode (a small grey dot located slightly off-center).

4) Fasten the cap for the student cell kit on the top of the 20 mL vial holding the blank standard solution. Carefully mount one carbon SPE onto the hand-grip. Use a second, partially cut, SPE to help mount the first electrode (see Figure 3). The cut SPE acts as a spacer, which insures proper electrical contact between the uncut SPE and the pins in the blue electrode connector.

5) Insert the hand-grip, together with the SPE, into the cap, and connect the mini-USB end to the port on the left side of the hand-grip (see Figure 3).

6) Select the **Cyclic Voltammetry** option from the **Experiments** menu, and then enter the experimental parameters shown in Figure 6. Note these parameters are suggested starting values for obtaining a background voltammogram. It may be necessary to make some minor adjustments in order to obtain a satisfactory voltammogram. In particular, it may be necessary to adjust the current range to optimize the appearance of the voltammogram.

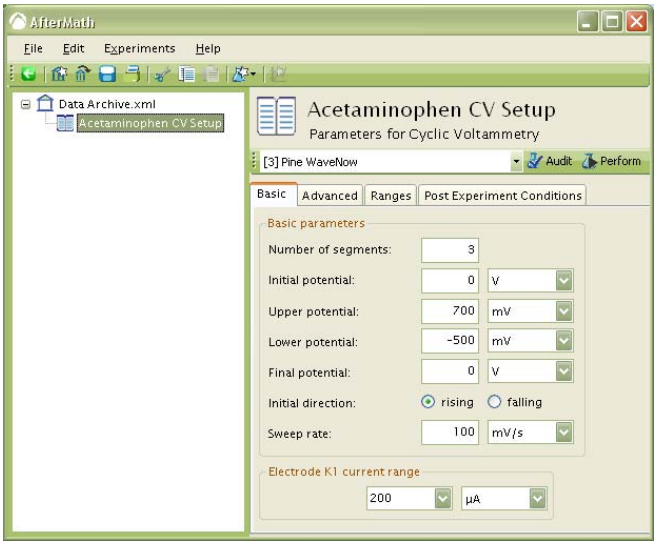


Figure 6: Configuring the Cyclic Voltammetry Parameters

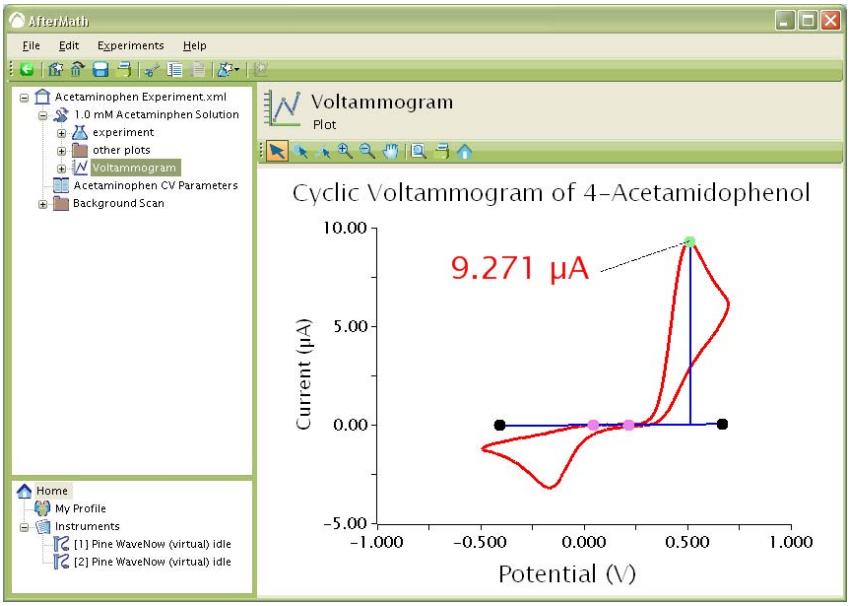


Figure 7: Example Cyclic Voltammogram of 4-Acetamidophenol in Buffer Solution

7) Once the experiment settings have been chosen, click on the "Perform" button to initiate the experiment. You may wish to repeat the experiment using different current ranges until an optimum range is found. Alternately, you can use the "Auto" current range and allow the software to select the optimal range. Note that you may observe small "glitches" in the data if you choose the auto-ranging option.

8) The "background" cyclic voltammogram should be relatively featureless (other than random sub-microampere noise) and exhibit no significant peaks. The overall background current should be less than 500 nanoamperes. If significant peaks are apparent, then the buffer, the glassware, and/or the electrode surface are likely contaminated. Consult with your instructor if excessive or unusual background current is observed.

9) After acquiring a satisfactory background voltammogram, save the data archive to the disk so that you do not lose your data. Give the archive a filename which is meaningful to you (such as "Acetaminophen Experiment"). Save the archive file to the disk frequently as you continue to acquire more results.

10) After each measurement using a SPE, rinse it with saline solution, and dry it either by gently blotting with a piece of paper towel or by blowing a stream of dry nitrogen over the electrode surface.

11) Note that each time you use a new SPE card, you should repeat this procedure and obtain a new background cyclic voltammogram. In this way, you can verify that the surface of the SPE has not be inadvertently contaminated by mishandling or touching the surface of the electrode.

C. Voltammograms of Standard Solutions

12) Create a new folder in your data archive called "Standard Solutions" by right-clicking on the data archive (left hand pane), and choosing the "New => Folder" menu option. Use the mouse to drag the background voltammogram and the CV parameters into this new folder.

13) Following the above protocol (Steps 6 to 11), obtain a cyclic voltammogram for each of the five remaining standard solutions that you have prepared. Each voltammogram should exhibit a prominent anodic peak near 0.5 volts and a smaller cathodic peak near -0.2 volts (see Figure 7).

IMPORTANT: Be sure to perform this series of measurements starting from the solution with the lowest concentration and systematically moving to higher concentrations.

14) After each voltammogram is obtained, be sure to rename it with a name that helps you to remember the corresponding solution concentration. Move each voltammogram into the "Standard Solutions" folder that you created in step (12). Keeping your data well-organized is helpful when analyzing it later.

NOTE: Because some of the reaction products can foul the surface of the SPE electrode, it is important that you only perform just one CV measurement per standard solution. This will minimize the gradual

deterioration of the SPE surface. Thus, try your best to verify all CV setup parameters before you press the "Perform" button. Set the "Electrode K1 current range" to 10 µA for all CV measurements, and use the same sweep rate (100 mV/s) for all solutions. When a solution in a vial is not in use, be sure to keep the vial securely capped.

15) Obtain a voltammogram of the Diluted Elixir Test Solution using a fresh SPE card. Remember to obtain a background scan (steps 6 to 11) for the fresh SPE card before using it to measure the voltammogram of the diluted elixir solution. Don't forget to save your archive of results to the disk periodically.

Data Analysis

A. Peak Height Measurements

The first step in analyzing your results is to obtain the peak height data from the voltammograms. The peak height is proportional to the acetaminophen concentration, and thus will be used to construct the calibration curve.

1) The AfterMath software provides an easy way to measure the height of peaks in a cyclic voltammogram (see Figure 7). By placing a special Peak Height tool on each voltammogram, you can measure the peak current for each of the five standard solutions.

HINT: To add a Peak Height tool to a voltammogram, select the curve using the left mouse button. Then, use the right mouse button to choose the "Add Tool => Peak Height" menu option. Next, move the five control points to their approximate locations as shown (see Figure 7).

HINT: You can zoom into the region near the two baseline control points (by pressing the button). This makes it easier to position the two baseline control points on the portion of the curve that is relatively flat (see Figure 8). The software will automatically use data points between the two control points to fit a straight line using the least square method, and this best fit becomes the baseline. You can also zoom into the region near the top of the peak to move the peak position control point to the highest data point.

HINT: After zooming in on a particular featrure, you can press the "home postion" button, to go back to the view of the entire voltammogram.

2) Using the Peak Height tool as described above, measure all peak currents for all of the standard

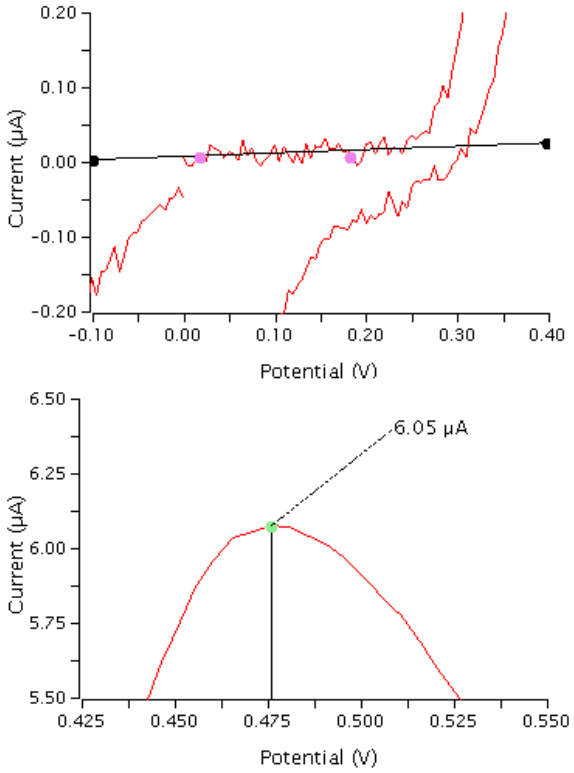


Figure 8: Zooming Detail with Peak Height Tool

solutions and also measure the peak current for the voltammogram obtained using the Diluted Elixir Test Solution. Record all of these results in your notebook.

B. Calibration Curve

3) Using the anodic peak height data, prepare the calibration curve of peak current versus acetaminophen concentration. This can be done using a piece of graph paper, or more preferably, a spreadsheet program such as Microsoft Excel.

4) Use the linear least squares method to fit a straight line to the data on the calibration curve.. Calculate the slope and the intercept of best straight line that fits the data, and write down the equation that correlates the peak current with the acetaminophen concentration.

C. Concentration in Children's Elixir

5) Using the equation for the best fit line, compute the acetaminophen concentration in the Diluted Elixir Test Solution.

6) Next, take into account the dilution of the original elixir (by the instructor and by the student) during the various preparation steps. After taking these dilutions into account, compute the acetaminophen

concentration in the original elixir (in grams per liter, using three significant figures).

7) Convert the acetaminophen concentration listed on the manufacturer's label to grams per liter (using three significant figures).

8) Assuming the manufacturer's label gives the "correct" concentration, what was the percent error in your result?

Report Questions

1) List at least two reasons that are outside of your control that could explain why your result differs from that listed on the manufacturer's label.

2) How would your results have been affected if you had failed to measure the anodic peak current against the proper background baseline?

3) Examine the peak height shown in Figure 7. Given that the oxidation of acetaminophen involves the removal of two electrons from the molecule, how many molecules per second must be oxidized in order to produce the observed peak current?

Reprints

Reprints of this document are available upon request from Pine Research Instrumentation, Inc., 5908 Triangle Drive, Raleigh, North Carolina 27617 (www.pineinst.com/echem)

References

1. Van Benschoten, J. J.; Lewis, J.Y.; Heineman, W. R.; Roston, D. A.; Kissinger, P. T. *J. Chem. Educ.* **1983**, *60*, 772-776. "Cyclic Voltammetry Experiment".

2. Potter, D. W.; Miller, D. W.; Hinson, J. A. *J. Biol. Chem.* **1985**, *260*, 12174-12180. "Identification of Acetaminophen Polymerization Products Catalyzed by Horseradish Peroxidase".

Instructor's Notes

A. Children's Tylenol Samples

Solid acetaminophen tablets may be used as suggested by Ref 1. However, it is usually difficult to get a tablet to dissolve completely (perhaps due to insoluble binding agents in the tablet). Using a liquid form of acetaminophen is much easier.

Measuring the volume of the viscous Tylenol accurately presents a challenge. Therefore, it is best to weigh the Tylenol sample during the preparation of the stock solution, and calculate the sample volume using its density. Before the lab starts, the instructor should measure the density by weighing the mass of children's Tylenol in a 100 mL volumetric flask or graduated cylinder. When pouring the liquid, try not to trap air bubbles into the liquid. The density of the children's Tylenol is typically 1.31 g/mL. The acetaminophen concentration in the elixir is about 32 g/L.

B. The Effect of Buffer pH

The pH-dependent electrochemical behavior of acetaminophen has been discussed in the introduction section, and a lab designed to illustrate the use of CV in studying electrochemical reaction mechanisms has been reported in Ref. 1. As Figure 9 shows, acetaminophen undergoes a nearly irreversible reaction in moderately acidic media.

Electrode fouling is a serious problem in acidic media because, in addition to the simple oxidation reaction discussed earlier, acetaminophen also undergoes an electro-polymerization reaction, which produces redox-inactive oligomers/polymers that block further redox activity at the electrode surface. This electrode fouling problem makes it impossible to quantify the acetaminophen concentration with CV because the anodic peak height will decrease gradually as the effective electrode area decreases. In short, electrolyte media with nearly neutral pH are ideal for quantifying purposes.

C. Other Electrode Problems

The carbon SPEs used in this lab generally behave reasonably well as a substrate for CV studies. Occasionally, a voltammogram may exhibit sharp peaks of less than 1 μA magnitude. This phenomenon is not well understood, and it might be due to slightly hydrophobic surface of a new SPE, which prevents the electrode surface from fully wetted. A small amount (< 0.1%) of surfactant (such as Triton X-100) may be added to improve the wetting property of the SPE carbon surface. If students show background voltammograms with sharp and small peaks, ask them to repeat scans several more times before giving them new SPEs.

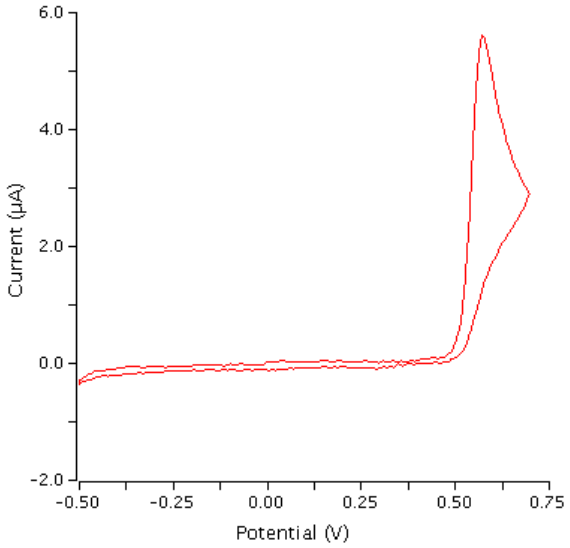


Figure 9: Voltammogram Acquired in 0.5M H_2SO_4

D. Related Analytical Methods

A titrimetric assay for acetaminophen with a colorimetric endpoint exists. The analyte is converted to 3-nitroacetaminophen using nitrous acid. Then, standard sodium hydroxide solution is used to titrate to the endpoint. The endpoint is signaled by the appearance of the yellow 3-nitroacetaminophen anion (λ = 430 nm). It may be of interest to have students use this method to confirm their electrochemical results.

Answers to Report Questions

Question 1

Several reasons for discrepancy between student results and the actual concentration are listed below.

• The formulation of any one particular batch of the Tylenol is likely to have an acetaminophen concentration different than that on the label. Presumably, drug manufacturing companies have good quality control, so the concentration is not likely to vary much from batch to batch.

• Similarly, the students rely on the acetaminophen standard prepared by the instructor.

• Using a fouled or dirty electrode (cf. previous descriptions on electropolymerization).

• Dilution errors

• The acetaminophen might bind to some other component of the Tylenol, thus the concentration of the "free" acetaminophen might be lower than the total.

• Other components of the Tylenol may be redox active and contribute to the anodic current measured using the Tylenol test solution.

Question 2

Failure to remove background current from the Tylenol Test Solution signal causes the reported concentration to be too high. Failure to remove background current from standard solution signals skews the calibration curve. The skewing is more pronounced at lower concentrations.

Question 3

The observed peak current is 6.05 μA. Using 96485 C/mol as the value for Faraday's constant, this current corresponds to a flow of 3.78×10^{13} electrons per second. This is chemically equivalent to oxidizing 1.89×10^{13} molecules per second.

