Experiment **1**

Determination of an Unknown: Measuring Acetaminophen using Cyclic Voltammetry

Acetaminophen is the active ingredient in numerous over-the-counter pain-relief medicines. As it is often recommended for children, acetaminophen is commonly sold as a liquid in various colors and flavors. A typical commercial sample has an acetaminophen concentration in the range of 30 to 100 g/L.

Medical professionals warn patients to be cautious when it comes to acetaminophen dosage. Billions of doses of acetaminophen are safely consumed every year, but accidents still occur. Thousands of people end up in the emergency room from accidental overdose, and a percentage of people die each year. This is due to a toxic byproduct generated by the breakdown of acetaminophen that occurs in the liver, as shown in the following reaction:

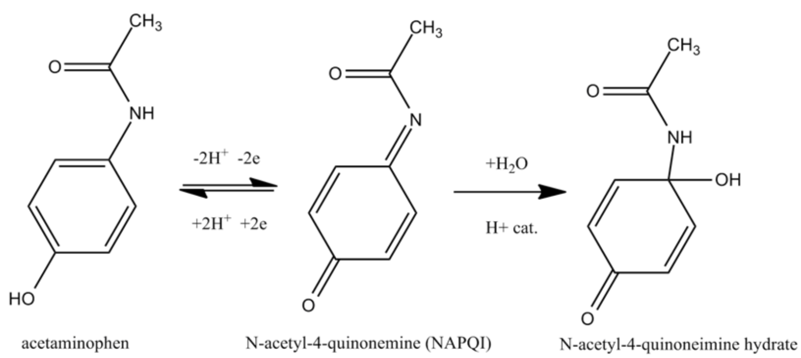

Figure 1

Acetaminophen is an electroactive molecule that can be oxidized to its quinone form, called N-acetyl-4-quinoneimine (NAPQI). NAPQI is formed in a two-electron, two-proton transfer process and is incredibly toxic to humans. In the presence of an acid catalyst, NAPQI is converted rapidly to a hydrate called N-acetyl-4-quinoneimine hydrate.

One method of determining the concentration of acetaminophen is cyclic voltammetry. Cyclic voltammetry is a very popular and often-used electroanalytical technique. A cyclic voltammogram (CV) is obtained by applying a linear potential sweep (that is, a potential that increases or decreases linearly with time) to a working electrode. As the potential is swept back and forth past the formal potential, E°, of an analyte, charge (electrons) flows between the working and reference electrodes. The rate of electron flow, called electrical current, is proportional to the concentration of analyte being oxidized or reduced, allowing cyclic voltammetry to be used in an analytical determination of concentration.

In this experiment, you will use cyclic voltammetry to determine the acetaminophen concentration in a sample of medication. You will then compare that value to the one provided by the manufacturer.

OBJECTIVES

- Use cyclic voltammetry, an electrochemical technique, to determine the acetaminophen concentration in a sample of medication.
- Compare the experimental concentration to the concentration provided by the manufacturer on the label.

MATERIALS

One of the following
- Chromebook, computer, or mobile device with Vernier Instrumental Analysis app[1]
- LabQuest 2 (software is pre-installed; v2.8.7 or newer required)[2]
- LabQuest 3 (software is pre-installed; v3.0.6 or newer required)[2]

Go Direct Cyclic Voltammetry System and stand
screen-printed electrode (SPE)
scintillation vial(s)
acetaminophen stock solution (1.0 mM) in slightly alkaline buffer
additional buffer
10 mL graduated cylinder or 1 mL volumetric pipet
(6) small volume beakers
unknown concentration of a sample of children's acetaminophen medication suspension
paper towels or lint-free wipes
goggles
Graphical Analysis app (used for data analysis)[3]

PRE-LAB ACTIVITY

Starting with your 1.0 mM acetaminophen stock solution, calculate and describe how you would accurately prepare (via serial dilution) 25 mL each of the following solutions: 0.8 mM, 0.6 mM, 0.4 mM, 0.2 mM, and 0.0 mM. Note the solvent you will use for dilution.

PROCEDURE

Part I Solution preparation

1. Put on safety goggles.

2. Based on your pre-lab calculations, start with your 1.0 mM acetaminophen stock solution and accurately prepare 25 mL each of the following solutions: 0.8 mM, 0.6 mM, 0.4 mM, 0.2 mM, and 0.0 mM. Dilute with buffer. **WARNING**: *Acetaminophen, solid,* $CH_3CONHC_6H_4OH$: *Acute toxicity, oral. Harmful if swallowed. Do not eat, drink or smoke when using this product. Skin and serious eye damage, corrosion or irritation. Causes skin and serious eye irritation. May cause respiratory irritation. Product should be treated as a chemical and is not for consumption as it has been stored with other non-food-grade chemicals. Store in a locked poison cabinet.*

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[1] Instrumental Analysis v1.2 or newer required; download the most recent version for free at **www.vernier.com/ia**

[2] Download the most recent version of LabQuest software for free at **www.vernier.com/downloads**

[3] Vernier Graphical Analysis app is available as a free download at **www.vernier.com/ga**

Part II Voltammogram of acetaminophen solutions

3. Set up the Cyclic Voltammetry System by following the directions for your equipment:

   Instrumental Analysis

   a. Launch Instrumental Analysis.
   b. Connect the Go Direct Cyclic Voltammetry System (CVS) to your device via USB or Bluetooth wireless technology. If using Bluetooth, click or tap Connect an Instrument, connect to your CVS, and click or tap Done.
   c. Click or tap Voltammetry.
   d. Set the CV Profile settings to the values in Table 1.

   LabQuest

   a. Connect the Go Direct Cyclic Voltammetry System (CVS) to your device via USB or Bluetooth wireless technology. If using Bluetooth, tap the Sensors menu and choose Wireless Device Setup►Go Direct. Select your instrument and tap OK.
   b. Choose Data Collection from the Sensors menu.
   c. Set the CV Profile settings to the values in Table 1. Make sure the Voltammetry mode is set to Cyclic Voltammetry.

Table 1	
Number of segments	3
Initial potential (mV)	0
Switching potential 1 (mV)	750
Switching potential 2 (mV)	–500
Final potential (mV)	0
Sweep rate (mV/s)	100
Current range	Medium (±100 μA)

4. Insert a screen-printed electrode (SPE) into the SPE connector on the Cyclic Voltammetry System (see Figure 2).

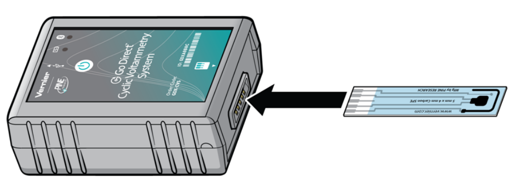

Figure 2

5. Fill the scintillation vial about halfway full (~10 mL) with your 0.0 mM solution. Insert the scintillation vial into the clip on the stand. Carefully guide the Cyclic Voltammetry System, with SPE attached, downward into the vial and snap the instrument into place (see Figure 3).

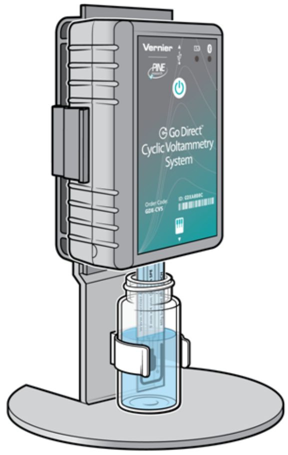

Figure 3

6. Start data collection. When data collection is complete, examine the background cyclic voltammogram. The voltammogram should exhibit no significant peaks (other than random sub-microampere noise). The overall background current should be less than 500 nA. If significant peaks are apparent, then the buffer, the glassware, and/or the electrode surface are likely contaminated. Consult your instructor if excessive or unusual background current is observed.

7. Name your sample appropriately.

8. Analyze the voltammogram by selecting the anodic peak (i.e., the peak to the right of V = 0 mV).
   - Instrumental Analysis: Click or tap Graph Tools, , and choose View Statistics. Record the Max value as the peak current in Table 2.
   - LabQuest: Choose Statistics from the Analyze menu. Record the Max value as the peak current in Table 2.

9. Carefully remove the Cyclic Voltammetry System from the stand by pulling back on the top tab (see Figure 4).

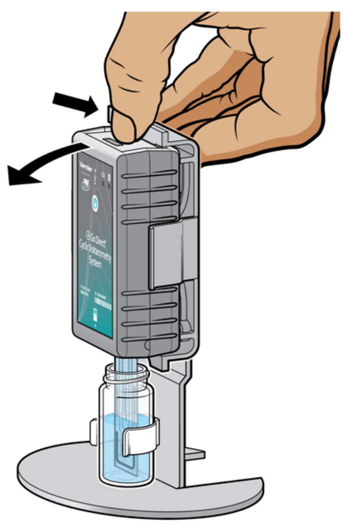

Figure 4

10. With the SPE still inserted in the Cyclic Voltammetry System, rinse the SPE with buffer solution, and dry it by *gently* blotting the electrode surface with a piece of paper towel. Take care when drying the SPE; rubbing too forcefully can damage the electrodes.
    Important: Do not invert the Cyclic Voltammetry System with a damp SPE attached. You want to avoid getting liquid inside the SPE connector.

11. Discard the 0.0 mM solution in the scintillation vial. Fill the vial approximately halfway full with the 0.2 mM solution. Insert the vial into the stand. Replace the Cyclic Voltammetry System with the SPE attached in the stand.

12. Start data collection. The voltammogram should exhibit a prominent anodic peak near 0.5 V and a smaller cathodic peak near –0.2 V. When data collection is complete, name your sample appropriately.

13. Repeat Step 8 to analyze the voltammogram and record data in Table 2.

14. Repeat data collection and analysis for the remaining solutions and your unknown commercial sample of children's acetaminophen medication suspension. **Hint**: To avoid contamination, proceed from the least concentrated sample to the most concentrated.

15. When you are finished collecting all of your data, save or export your data as instructed. Dispose of your solutions and SPE as instructed.

DATA TABLE

Table 2

Acetaminophen concentration in solution (mM)	Peak current (μA)
0.00	
0.20	
0.40	
0.60	
0.80	
1.00	
Unknown	

DATA ANALYSIS

1. Launch Graphical Analysis app and select Manual Entry mode. Enter the data from Table 2 to create a graph of peak current versus acetaminophen concentration.

2. In Graphical Analysis app, click or tap Graph Tools, , and select Apply Curve Fit. Apply a linear fit to fit the calibration curve to the data. Use this information to determine the

concentration in the medication test solution. Make sure you consider all the dilutions made during the preparation of the test solution.

3. In Instrumental Analysis app or LabQuest, examine the peak current of your most concentrated sample. 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?

4. From your data analysis, compare your determination of acetaminophen concentration with the value given on the label of the commercial medication. Assuming the manufacturer's label is accurate, what was the percent error in your result?

EXTENSION

Using the standard sample with the highest concentration of acetaminophen and a new SPE, run several voltammograms. With each run, modify the scan rate. Determine the relationship between scan rate and peak current. Justify your response.