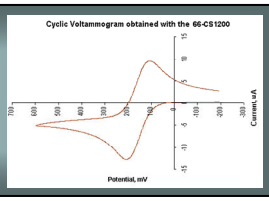

7. Ascorbic Acid (Vitamin C): A Cyclic Voltammetric Study of its Oxidation at a Glassy Carbon Electrode

PURPOSE: To learn how to prepare an active glassy carbon electrode for the oxidation of ascorbic acid that undergoes an irreversible chemical reaction (*c* step) following the electron transfer (*e* step).

BACKGROUND: Ascorbic acid (AA) or vitamin C is widely known as an antioxidant and a free radical scavenger. It is also important in helping to produce collagen, a protein needed in the development and maintenance of bones, cartilage, joint linings, skin, teeth, gums and blood vessels. It's reputed antiviral properties to prevent the common cold remains controversial.

AA is a water-soluble vitamin found mainly in fruits and vegetables, particularly green leafy veggies, citrus fruits, tomatoes, guavas, melons and berries. Because these were lacking in the diet of early day sailors, scurvy was common, often resulting in death. It is a white crystalline powder with a molecular formula of $C_6H_8O_6$ and a F.W. of 176.12. The recommended daily intake is 200 – 500 mg per day [ref. 1,2].

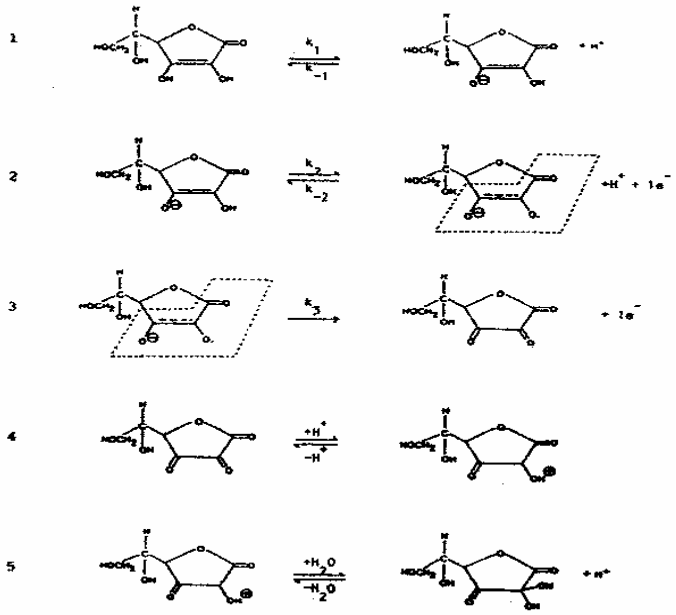

Figure 1. Proposed mechanism for the electrooxidation of ascorbic acid in acid or neutral solution [ref. 3].

The oxidative mechanism for AA, as proposed by Ruiz [ref. 3], for pH 8 or less is shown in Figure 1. The electron transfer involves the deprotonated anion (step 1) that is oxidized in a one electron, one proton reaction (step 2) to the radical anion. A subsequent fast one electron irreversible oxidation takes the anion to dehydroascorbic acid (DHAA, step 3). It is electro-inactive. DHAA is rapidly protonated and then dehydrated to 2,3-diketogluconic acid (steps 4 and 5).

Cyclic voltammetry (CV) is a convenient electrochemical method to examine the oxidation of AA at a glassy carbon (GC) electrode that has been "activated" to different degrees. That is, the peak height (Ip) and the peak potential (Ep) are very dependent on the state of the GC surface. Besides contamination, the surface may contain carbon-oxygen functionalities from prolonged exposure to air and moisture. With polishing, surface contaminants and functionalities may be minimized to give a "clean" carbon surface at which AA undergoes a relatively fast electron transfer process, albeit the deprotonated anion being the electroactive species. The 1st pKa of AA is 4.17 and the 2nd is 11.57. Thus, the parent AA is the predominant species in solution at pH 2 where experiments will be run. Since steps 1-3 are fast on the

time scale of scan rates used, and the product of reaction 3 is irreversible, only the forward 2 electron, 2 proton CV wave is observed. The reverse cathodic wave, attributable to the reduction of the radical anion (product of step 2) is seen only at high scan rates where the time window competes favorably with the kinetic rate (~ 10^3 s^{-1}) of its removal. You may wish to run CVs at high scan rates (e.g., 10 V/s or more) if your potentiostat has the capability to scan at these high rates to see if you can "capture" this radical anion. Details of AA's electrochemical oxidative mechanism are reported in references 3 and 4. Example CV waves at three different levels of GC electrode activity are shown in Figure 2.

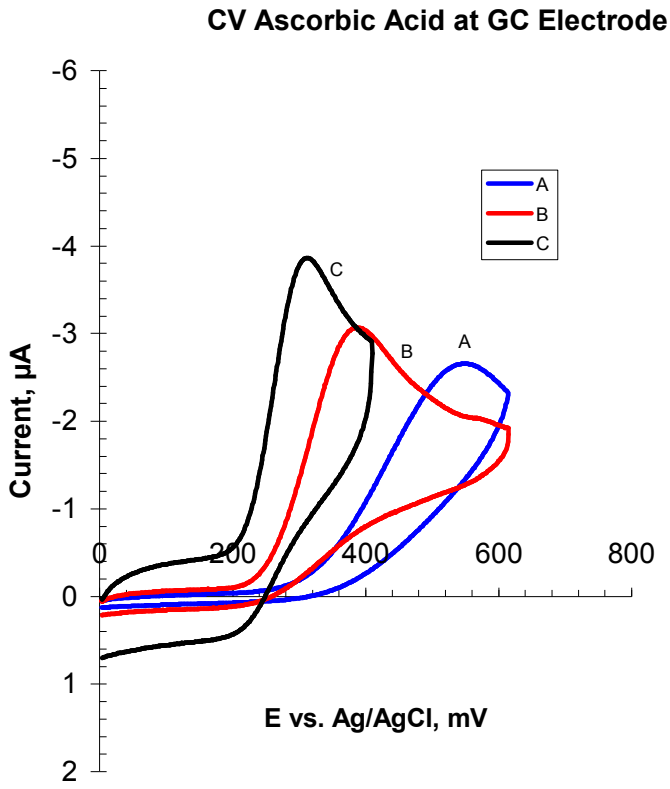

Figure 2. CV of ascorbic acid oxidation at different levels of GC electrode activation. Trace A is an electrode used without any pretreatment; B is the same electrode after a cursory polish with 0.5 µm alumina and washed with pure water; C is the electrode after light polishing on Grit 0000 paper followed by polishing with 0.5 µm alumina on a glass plate and washed with pure water.

The CV with the lowest Ep value (i.e., trace C in Figure 2) has a large background current indicative of a higher effective surface area, possibly due to micro-graphitic particles on the surface left from the polishing process. One of the most pristine surfaces is produced by a vacuum heat treatment that gives rise to a well-defined CV with a peak at ~ 240 mV [ref. 4].

In biological fluid samples, like urine, the presence of uric acid interferes due to its oxidative potential being similar to AA. Liquid chromatographic with electrochemical detection (LCEC) is an effective method to separate and determine AA and uric acid independently. Cyclic voltammetry has been proposed as a method to evaluate the antioxidant capacity of AA in biological samples such as blood plasma, tissue homogenates and plant extracts [refs. 5-7].

In this experiment CV scans will be conducted after various pretreatments of GC electrode. When a reproducible method is found, calibrations with known AA solutions will be run prior to determining amount of AA in a fruit juice sample.

EXPERIMENT

Equipment

- An analog or computer-controlled potentiostat with appropriate data acquisition capability (ask you laboratory instructor about potentiostat and data recorder).
- Electrochemical cell
- 1 - 3mm diameter flat tipped glassy carbon electrode
- Ag/AgCl reference electrode
- Pt auxiliary electrode
- Electrode polishing kit

Chemical Solutions

- 100 ml of 10 mM AA in 0.10 M phosphate buffer at pH 2 containing 0.5 g/L Na_2EDTA to prevent the oxidation of AA. It is recommended that the solution be deaerated to remove oxygen prior to adding AA. Store solution in a refrigerator when not in use. It is important to use high purity water and clean glassware. Record the concentration to three significant figures.
- 250 ml of stock 0.1 M phosphate buffer at pH 2.
- 50 ml of 2.0 mM AA in pH 2 phosphate buffer by dilution of stock solutions 1 and 2. Keep tightly sealed when not in use. Prepare daily from stock.
- 50 ml test sample by diluting 1:20 commercial fruit drink with the pH 2 phosphate buffer. Use immediately.

Procedure

1. Recommend the use of a 3.0 ml cell, with a 1.0 mm or 3.0 mm disk GC electrode, an Ag/AgCl reference and a Pt auxiliary electrode. It is recommended that rubber gloves be used to handle the cell and electrodes.

2. Run #1: Fill the cell so that the electrodes are immersed in the 2.0 mM AA solution. Connect the electrodes to the potentiostat. All scans will be from 0.0 V to +0.6 V and back to 0.0 V at scan rates of 20 mV/s, 100 mV/s and 200 mV/s. Use the GC electrode without polishing (this will serve as the "inactive" electrode). Wait 2 minutes between scans to allow the concentration at the electrode surface to equilibrate with the bulk solution.

3. Run #2: Repeat after removing and polishing the GC electrode with 0.05 μm alumina on a clean glass plate and rinse with pure water prior to use. Please refer to "Activation of Glassy Carbon Electrode" that is available as a Technical Note. Add the scan rates of 200 mV/s and 1.0 V/s and set the number of scans to 2. If the CV wave at 100 mV/s does not exhibit a sharp peak, as seen in Trace C of Figure 2, it may be necessary to polish the electrode first on a fine grit 0000 paper followed by alumina.

4. Set the scan rate to 100 mV/s and obtain CV of AA at concentrations of 0.50 mM, 1.0 mM, 2.0 mM and 4 mM in pH 2 phosphate buffer. Run a background CV of the buffer without AA at these scanning rates to correct for the peak heights.

5. Run duplicates of the fruit juice sample and determine its concentration by comparing the peak height to a calibration plot of concentration versus peak height, as determined in step #3. If the CV peaks are not sharp and well defined, re-polish with alumina to activate the electrode.

6. It is recommended that you save all CV data on the hard drive or memory card with file names or in a folder with your name for future reference. If data are saved as an ASCII file, transfer data to a Microsoft EXCEL program and re-plot the data files. Excel gives you options to make different formatted graphs.

REPORT (Data, analysis and discussion):

Consult with your lab instructor about contents and format. Suggested contents include graphs or plots, calculations, and answers to questions with supporting discussions.

1. Show cyclic voltammograms of AA at "inactive" and "active" GC electrode.

2. Graph of calibration plot of AA concentration vs. peak height.

3. Example cyclic voltammogram of fruit juice sample.

4. The concentration of AA in the fruit juice (how close is your value to that on the label).

5. Show background CV of buffer and a background corrected CV of AA (one example will suffice).

6. Discuss the scan rate dependence of peak height. Does it change according to a known relationship?

7. Discuss the reason for the absence of a reverse wave when the potential is scanned from +600 mV to 0.0 V.

8. Explain why AA serves as an antioxidant.

9. Find the literature value for the reversible E^o of AA. Is the value close to what you observe experimentally? What are three (or more) possible reasons for the observed differences?

REFERENCE:

1. http://www.medicinenet.com/script/main/art.asp?articlekey=12536

2. http://www.anyvitamins.com/vitamin-c-ascorbicacid-info.htm

3. G. Dryhurst, K. Kadish, F. Scheller, R. Renneberg, *Biological Electrochemistry*; Academic Press, New York, 1982; Vol. 1, pp. 256-277.

4. F. Hu, T. Kuwana, *Oxidative Mechanism of Ascorbic Acid at Glassy Carbon Electrode*, Anal. Chem., 58 (1986) 3235–3239.

5. S. Chevion, M. A. Roberts, M. Chevion, *The Use of Cyclic Voltammetry for the Evaluation of Antioxidant Capacity*, Free Radical Bio. & Medicine, 28 (2000) 860-870.

6. S. Chevion, E. M. Berry, N. Kitrossky, R. Kohen, *Evaluation of Plasma Low Molecular Weight Antioxidant capacity by Cyclic Voltammetry*, Free Radical Biol. & Medicine, Vol. 22 (1996) 411-421.

7. J. Psotova, J. Zahalkova, J. Hrbac, V. Simanek, J. Bartek, *Determination of Total Antioxidant Capacity in Plasma by Cyclic Voltammetry. Two Case Reports*, Biomed. Papers, 145 (2001) 81-83.

OPTIONAL EXPERIMENT

Vitamin C (i.e., ascorbic acid) dietary supplement 300 mg or 500 mg pills can be substituted in place of the fruit juice as the analytical sample. It is necessary to grind the pills to a powder and weigh the amount to give an estimated 1 – 2 mM solution in pH 2 buffer. Some filler material in the pills may be insoluble.