Cost-Effective Teacher

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Measuring Vitamin C Content of Commercial Orange Juice Using a Pencil Lead Electrode

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Cyclic voltammetry is an analytical technique in which current is measured while the voltage is varied in an electrochemical cell. The measured current is directly proportional to the concentration of the analyte. Cyclic voltammetry is carried out in an electrochemical cell consisting of a working, a reference, and an auxiliary or counter electrode *(1)*. The working electrode is usually made of a metal or carbon. The most common carbon electrode is made from glassy carbon (GC). Various forms of graphite have also been used as a working electrode. A homemade glassy polymeric carbon electrode has been described in this *Journal (2)*.

At the time of the Roman Empire, pencils contained a thin lead rod. In the late 1500s, the lead metal rod was replaced by graphite and subsequently by a graphite—clay mixture after 1795. It is worth noting that the inaccurate term, pencil lead, is still used to this day even though modern pencils contain no lead. Modern pencils are usually labeled with numbers and the letters B and H. A larger number indicates a harder (H; more clay) or blacker (B) pencil *(3, 4)*.

The use of pencil lead as a sensitive nitrate ion-selective electrode *(5)*, for a simple surface chemistry experiment *(6)* and for potentiometric titrations *(7)*, has been described in this *Journal*. The determination of vitamin C using a variety of methods has also been described in this *Journal*: gold electrodes modified with self-assembled monolayers *(8)*, biopotentiometric iodometric back-titration *(9)*, microscale weight titrimetry *(10)*, high-pressure liquid chromatography *(11)*, titration using 2,6-dichlorophenolindophenol and *N*-bromosuccinimide *(12)*, and a combination of iodometric and coulometric titrimetry *(13)*.

In this article, the use of pencil lead as a working electrode for the determination of vitamin C in commercial orange juice is described. The detection limit of the pencil lead electrode for the measurement of ascorbic acid is also determined.

Experimental Method

Several different kinds of pencils were tested to determine the pencil with electrochemical properties closest to those of a carbon electrode; this was done by measuring the electron transfer rate for a number of pencils. The pencils tested included types 5B, 3H, and HB. The electron transfer rate was determined from cyclic voltammograms of potassium ferricyanide, $K_3Fe(CN)_6$ (1 mM; Fisher Scientific), a benchmark redox reagent, solution in 1 M aqueous KCl (Fisher Scientific).

A calibration curve was constructed using standard additions of ascorbic acid to Sun-Rype orange juice. The concentration of ascorbic acid (Sigma-Aldrich) used was 0.878 mg/mL. Four standard solutions were prepared by measuring 0.00, 5.00, 10.00, and 15.00 mL of ascorbic acid solution into 50 mL volumetric flasks. A 25.00 mL aliquot of 100% orange juice was added to each volumetric flask. KCl, 3.73 g, was added to make the solutions 1 M in KCl as a supporting electrolyte. The solution was then diluted to volume using distilled water. Enough solution was transferred into an electrochemical cell for analysis. All solutions were purged with nitrogen prior to use. From the cyclic voltammograms obtained, the maximum peak current was determined and used to construct a standard addition calibration curve.

A titration of the orange juice was carried out using 0.0300 M iodine solution that was prepared by dissolving 0.757 g of I_2 (Fisher Scientific) mixed with 2.00 g of KI (Fisher Scientific) in distilled water in a 100 mL volumetric flask. The iodine solution was standardized by titrating with As_2O_3 (Fisher Scientific). Before use, the As_2O_3 was dried in an oven for 2 h at 110 °C. To dissolve the As_2O_3, the following procedure was used: 0.300 g of As_2O_3 was weighed into a 250 mL beaker, and 10.0 mL of 10.0 M NaOH (Sigma-Aldrich) added. After the solid dissolved, 100 mL of distilled water and 3 drops of phenolphthalein indicator (Shawinigan Chemicals) were added, followed by 6 M HCl (Fisher Scientific) dropwise, until the solution turned colorless. The solution was then quantitatively transferred to a 250 mL volumetric flask and diluted to the mark with distilled water.

The detection limit of the pencil electrode was determined by measuring 4.00, 8.00, 16.00, and 100.00 mL aliquots of an 0.816 mg/mL stock solution of ascorbic acid into 100 mL volumetric flasks and diluting to volume using distilled water.

Cyclic voltammetric measurements were performed in a sealed-bottom three-electrode cell in which the working electrode was a pencil lead, which was easily prepared by sharpening both ends of the pencil with a mechanical sharpener. An attempt was made to have a fairly constant surface area of exposed graphite on the working electrode. An alligator clip was used to connect the pencil to the potentiostat. A platinum auxiliary electrode and a silver/silver chloride reference electrode were used. The cell was connected to a potentiostat (model AFCBP1, Pine Instruments, Grove City, PA). Data were recorded on a computer using PineChem (Version 2.8.0) software.

Hazards

Potassium ferricyanide can generate a highly toxic gas (HCN) if mixed with acids. Potassium iodide and potassium chloride may be harmful if absorbed through skin and may also

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Table 1. Cyclic Voltammetric Peak Separations (ΔE_p) for Carbon and Pencil Electrodes

Electrode	Average ΔE_p/mV[a]
Glassy carbon (GC)	80
Highly ordered pyrolytic graphite (HOPG)	90
HB (#2) pencil	150
3H pencil	200
5B pencil	220

[a] Data using 1 mM $Fe(CN)_6^{3-/4-}$ voltammograms.

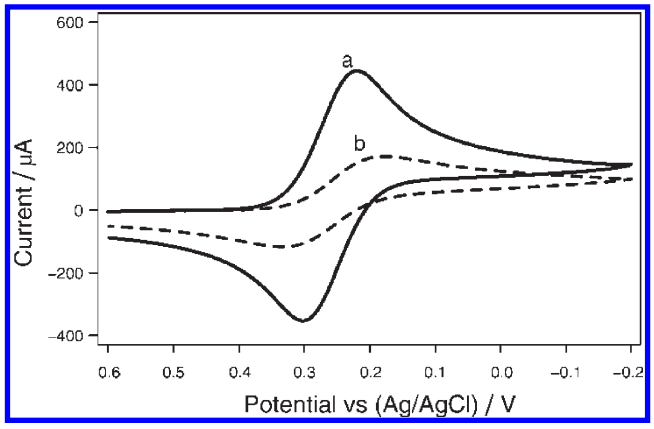


Figure 1. Cyclic voltammetry of 1 mM $Fe(CN)_6^{3-/4-}$ in 1 M KCl using a carbon electrode (curve a) and a HB (#2) pencil lead electrode (curve b). Scan rate: 100 mV/s.

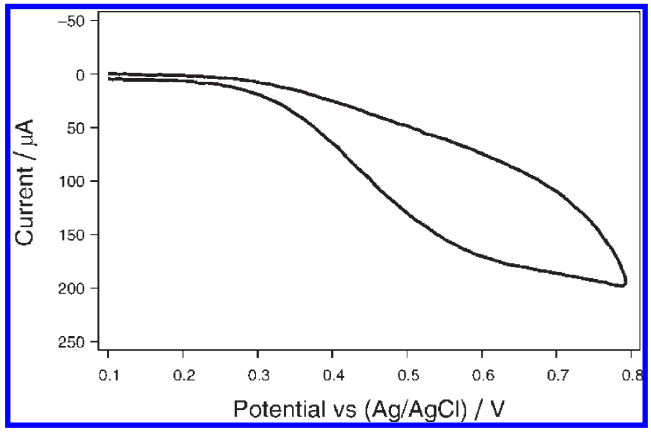


Figure 2. Cyclic voltammetry of a standard addition sample containing 15.00 mL of 0.878 mg/mL ascorbic acid, 25.00 mL orange juice, and 1 M KCl.

cause skin irritation. Arsenic trioxide is toxic and may cause cancer; it must be handled with care and disposed of properly. Sodium hydroxide, hydrochloric acid, and iodine are corrosive and can cause skin and eye burns, and their vapors can cause chemical burns to the respiratory tract. The use of goggles and gloves is recommended when handling all solutions.

Results and Discussion

Our results indicated (Table 1) that the HB pencil (also identified as #2 by a different labeling scheme) has electrochemical properties closest to those of a commercial carbon or graphite electrode. This was determined by comparing the peak potential separation (ΔE_p) of the cyclic voltammograms of 1 mM $K_3Fe(CN)_6$, as shown in Figure 1. The ΔE_p values can be converted to electron transfer rate constants using a published method *(14)*. The smaller the ΔE_p, the faster the rate of electron transfer of the pencil tested. An HB pencil was used in all subsequent experiments described in this article.

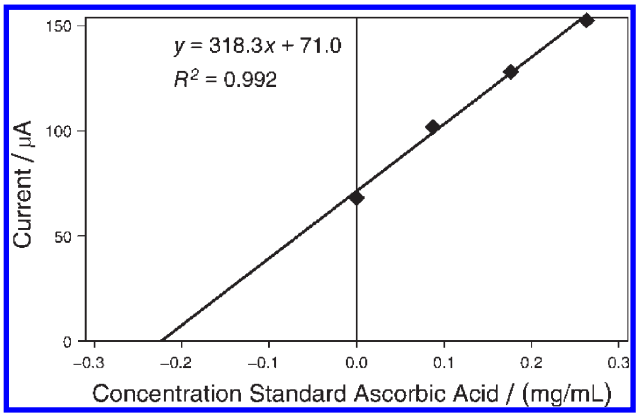
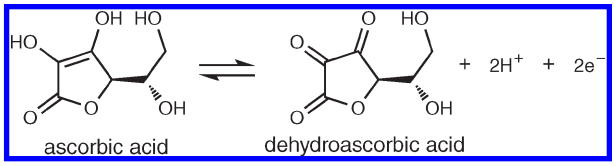


Figure 3. Calibration curve using standard additions of 0.878 mg/mL ascorbic acid in 25.00 mL orange juice.

The oxidation of ascorbic acid results in the formation of dehydroascorbic acid *(15)*:



A typical voltammogram for the oxidation of ascorbic acid is shown in Figure 2. The anodic peak corresponding to the oxidation of ascorbic acid has a maximum current, which is proportional to the concentration of ascorbic acid, at 550 mV. The continued increase in current observed at voltages beyond 550 mV is due to the oxidation of the solvent. Therefore, the current at 550 mV was measured for all subsequent voltammograms. A calibration curve of peak current versus concentration of added ascorbic acid was plotted. A typical calibration curve is shown in Figure 3. The calibration curve experiment was repeated three times. From the calibration curve, the average quantity of vitamin C was determined to be 107.9 ± 7.0 mg/250 mL of orange juice when using a pencil as the electrode, while the quantity of vitamin C was determined to be 97.7 ± 5.9 mg/250 mL using a commercial carbon electrode. There is no significant difference between the two average values based on the Student *t* test at the 95% confidence level. The total quantity of vitamin C in Sun-Rype orange juice is claimed to be 60 mg/250 mL on the manufacturer's Web site *(16)*. The same value, which is also the recommended daily intake of vitamin C *(17–19)*, was also provided by a representative of Sun-Rype Products Limited, the producer of the orange juice.

To check the accuracy of our method, we determined the quantity of vitamin C in Sun-Rype orange juice by titration using a standardized iodine solution. Three titrations were carried out. The quantity of vitamin C was found to be 103.3 ± 0.2 mg/250 mL.

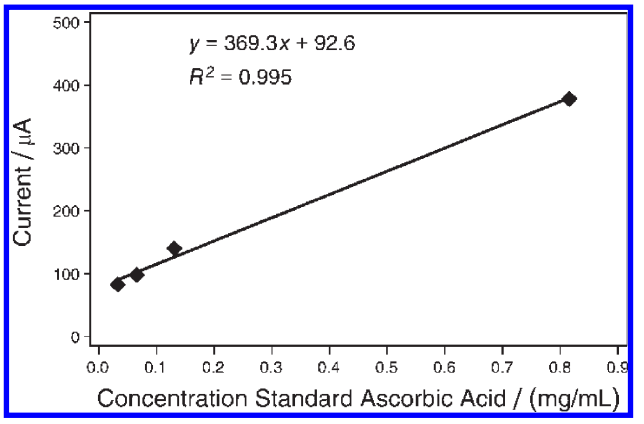


Figure 4. Calibration curve for the determination of the detection limit of standard ascorbic acid.

The value is not significantly different to that obtained using the voltammetry method. This confirms the accuracy of the data obtained using the voltammetric method. Owing to this validation of our experimental method, we think that the commercial Sun-Rype orange juice contains more vitamin C than the quantity, 60 mg/250 mL, stated on the label.

The lowest concentration of ascorbic acid that could be measured using the pencil electrode and still produce a linear calibration curve was determined to be 0.0326 mg/mL. Concentrations lower than 0.0326 mg/mL resulted in a nonlinear calibration curve. The results are shown in Figure 4.

Conclusions

In this experiment, we have shown that cyclic voltammetry employing a pencil lead can be used to accurately determine the quantity of vitamin C in orange juice. The vitamin C concentration obtained using a pencil lead as the electrode (<$1) is comparable to that obtained using a commercial glassy carbon electrode (~$200). According to our results, the quantity of vitamin C contained in Sun-Rype orange juice tested is about 1.5 times the recommended daily intake for a healthy adult. Ingestion of modest excesses of vitamin C is not a health concern because surplus quantities of this water-soluble vitamin are readily excreted *(20, 21)*.

Our results also show that the pencil electrode is suitable for measuring ascorbic acid quantities that are typically found in commercial orange juices. We are conducting further experiments with the aim of improving the detection limit of the pencil electrode.

It is our intention to compare the utility of pencil electrodes to commercial electrodes in other electroanalytical experiments. In the meantime, we hope that the experiment described in this article can be adopted as a laboratory exercise in analytical chemistry classes. The lab would introduce students to fundamental electrochemistry techniques, sample analysis using the method of standard addition, as well as statistical data analysis.

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