Studying Current–Potential Curves Using a Bipotentiometric Iodometric Back-Titration for the Determination of Ascorbic Acid in Fruits and Vegetables

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In instrumental analysis courses students are exposed to an impressive array of methods that allow them to solve analytical problems. It then becomes a challenge for the students to intelligently choose among the many possible techniques that provide the required analytical information for a sample. Understanding the fundamental principles of each method allows students to select the best technique.

Applications of electrochemical techniques have been the subject of considerable interest in recent years. However, in our experience, principles of voltammetry are not appealing enough to encourage students to a deep study of these techniques. The aim of this laboratory experiment is to draw students' attention to voltammetric methods, in particular to the study of current–potential curves, stressing their potential applicability in areas of current interest, such as food quality control. The determination of ascorbic acid in biological samples is a concrete application of voltammetric analysis that shows the use of electrochemical techniques in an area of interest to the students. This experiment is proposed for students of chemistry, biochemistry, and health related sciences, during an instrumental analysis course.

Students gain insight into a classical redox titration and the advantages of back-titration. They are introduced to electroanalytical methods by a bipotentiometric titration with an emphasis on theoretical current–potential curves. This should help students better understand the voltage change occurring at a particular electrochemical cell and infer cell behaviors in which other species are involved. If time permits, students can also analyze duplicates of the samples by direct iodometric titration and compare the results with respect to those obtained by the back-titration. This additional experiment will make the advantages of the back-titrations readily evident.

Background

Interest in food chemistry is increasing at many universities and colleges. Several analytical applications for the determination of vitamin C in chemistry courses have been reported in this *Journal (1–4).* A variety of methods for determining ascorbic acid (AA) in fruits and vegetables are currently being used: spectroscopic (colorimetric and fluorometric), chromatographic (HPLC), and electrochemical methods *(5–9).* Spectroscopic and chromatographic methodologies require ultracentrifugation or filtration to remove particles in suspension resulting from homogenization of the sample during vitamin extraction. Those steps are time consuming and increase the chances of AA oxidation. Several electroanalytical methods avoid some laborious steps when preparing the sample. However, most of these methods are based on the use of specific electrodes that are not frequently available at a teaching laboratory *(10–13).*

Iodometric techniques have been largely used to quantify weak reductants that cannot be determined by the commonly used strong oxidizing primary standard, potassium dichromate *(14, 15).* Iodometry has been used to quantify AA in citrus juices and soluble samples of vitamin C *(16, 17).* Many of these methods titrate the AA directly with triiodide solution. The potential drawback to this procedure is that AA is relatively unstable and will rapidly oxidize in the absence of titrant. We have presented and validated a method for determining AA in fruits and vegetables that combines an iodometric back-titration with voltammetric (bipotentiometric) end point detection *(18).* In that work, we performed specificity assays and demonstrated that the presence of other reducing compounds occurring in natural samples produces negligible interference. Furthermore, in that article, we compare the results obtained via a voltammetric titration with those obtained using HPLC analysis; statistical analysis using one-way ANOVA showed no difference between both methods *(18).*

The advantages of the bipotentiometric iodometric back-titration method are as follows: (i) it avoids the usage of preserving solutions to extract the vitamin since the sample processing takes no longer than three minutes; (ii) it bypasses laborious sample preparation steps such as ultracentrifugation or filtration; (iii) the iodometric back-titration causes the AA to react very rapidly with triiodide, thus avoiding the risks of oxidation caused by the dissolved oxygen; (iv) the end point is easily detected in a few minutes; and (v) this technique employs platinum electrodes routinely found in many laboratories and allows efficient quantification of AA with low-cost reagents and equipment.

Experimental

Equipment

- Potentiometer with low current option or voltamperometer or amperometer.
- Knife homogenizer
- Double platinum electrode
- Automatic magnetic stirrer

Reagents

- 0.1500 N $K_2Cr_2O_7$
- 5% w/v KI
- H_3PO_4 (pH = 0.5)
- 0.05 N $Na_2S_2O_3$

Procedure

Detailed procedures to prepare the triiodide solution, to standardize the sodium sulfate solution, to prepare the fruits and vegetables, and to conduct the voltammetric titration are available in the Supplemental Material.[W]

Discussion

A known excess of triiodide is generated by reaction of dichromate with iodide solution:

$$9I^- + Cr_2O_7^{2-} + 14H^+ \rightarrow 3I_3^- + 2Cr^{3+} + 7H_2O \quad (1)$$

The fruit or vegetable sample is homogenized in water with a knife homogenizer and immediately added to the triiodide solution. The AA ($C_6H_8O_6$) present in the sample reacts with the generated triiodide consuming part of it, to give dehydroascorbic acid ($C_6H_6O_6$) and iodide:

$$C_6H_8O_6 + I_3^- \rightarrow C_6H_6O_6 + 2H^+ + 3I^- \quad (2)$$

The excess triiodide is quantified with a previously standardized sodium thiosulfate solution:

$$I_3^- + 2S_2O_3^{2-} \rightarrow 3I^- + S_4O_6^{2-} \quad (3)$$

This titration is monitored by means of a voltammetric technique using a double platinum electrode. The potential difference between both electrodes, ΔE, is registered against the added volume of titrant (mL of $S_2O_3^{2-}$) and the equivalence point volume is obtained graphically from a ΔE versus volume of $S_2O_3^{2-}$ plot. A theoretical titration curve is shown in Figure 1. A better understanding of this titration curve can be achieved by analyzing the schematic current–potential curves depicted in Figure 2 for the anodic and cathodic half-cell reactions that depend on the species present at each stage of the titration *(19–21)*. Consider eq 3, where titrated I_3^- reacts with titrant $S_2O_3^{2-}$. Before adding the titrant the

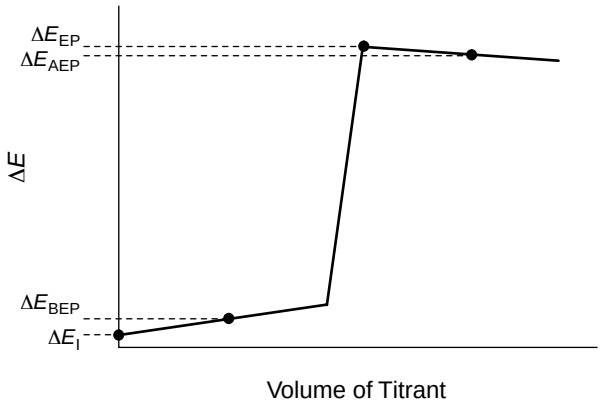

Figure 1. Theoretical titration curve of a triiodide solution with thiosulfate: ΔE_I—voltage across the cell at the beginning of the titration; ΔE_{BEP}—voltage across the cell at a selected volume before the equivalence point; ΔE_{EP}—voltage across the cell at the equivalence point; ΔE_{AEP}—voltage across the cell at a selected volume after the equivalence point.

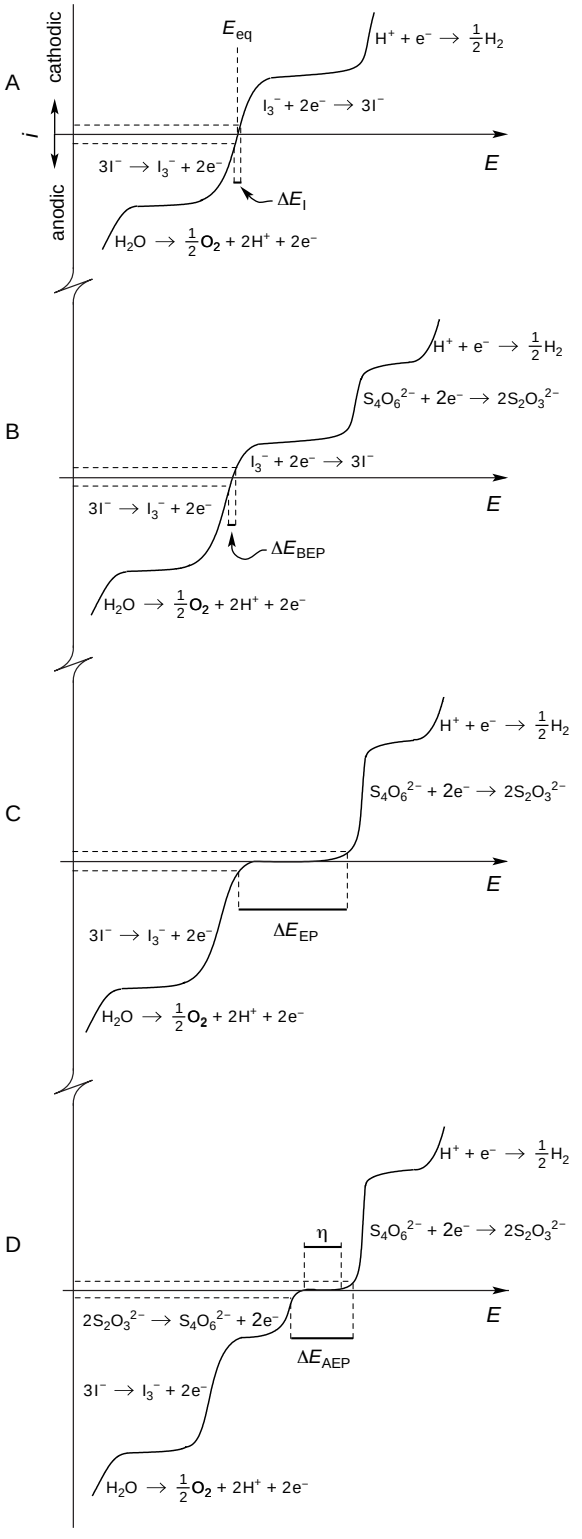

Figure 2. Schematic current–potential curves for anodic and cathodic half-cell reactions during the titration: (A) before addition of titrating agent, (B) before the equivalence point, (C) at the equivalence point, and (D) after the equivalence point. ΔE_I—initial cell voltage; ΔE_{BEP}—voltage across the cell before the equivalence point; ΔE_{EP}—voltage across the cell at the equivalence point; ΔE_{AEP}—voltage across the cell after the equivalence point; E_{eq}—equilibrium potential for the couple I_3^-/I^-; η—overpotential for the irreversible couple $S_4O_6^{2-}/S_2O_3^{2-}$.

species present are I_3^- and I^-. The expected electrode processes are iodide oxidation (anode) and triiodide reduction (cathode). The current–potential curve for the reversible I_3^-/I^- couple is shown in Figure 2A. Notice that the anodic branch contacts the *x* axis at a potential close to the equilibrium potential, E_{eq}, given by the Nernst equation and the cathodic branch rises at a potential near E_{eq}. At this point, it is apparent that the initial voltage across the cell, ΔE_I, corresponds to a few millivolts (Figure 2A).

As the titration proceeds, $[I_3^-]$ decreases and $[I^-]$ increases. Hence, the limiting anodic current for iodide oxidation increases, while the limiting cathodic current decreases. However, the cell voltage before the equivalence point, ΔE_{BEP}, remains at low values (Figure 2B). When $[I_3^-]$ is negligible, the cathodic limiting current is virtually equal to zero, thus increasing the cell voltage. The species present at the equivalence point are I^- and $S_4O_6^{2-}$, the feasible electrode reactions being iodide oxidation at the anode and reduction of $S_4O_6^{2-}$ at the cathode. The voltage across the cell at this point, ΔE_{EP}, is therefore determined by the difference between the cathode potential for reduction of $S_4O_6^{2-}$ and the anode potential for iodide oxidation (Figure 2C). As can be seen, the cell voltage is much larger than before the end point.

After the end point, further additions of titrant produce $S_2O_3^{2-}$ in the solution. The new current–potential curve at this stage corresponds to the irreversible couple $S_4O_6^{2-}/S_2O_3^{2-}$ where the cathodic and anodic branches are separated by an overpotential, η (Figure 2D). Notice that the cell voltage, ΔE_{AEP}, decreases because the anodic branch for $S_2O_3^{2-}$ oxidation is to the right of the anodic branch for the couple I^-/I_3^-. Further additions of titrant $S_2O_3^{2-}$ will cause no significant differences in the cell voltage. The changes in cell voltage from ΔE_I to ΔE_{BEP}, ΔE_{EP}, and ΔE_{AEP} clearly explain the shape of the titration curve obtained in this experiment (Figure 1 and 2).

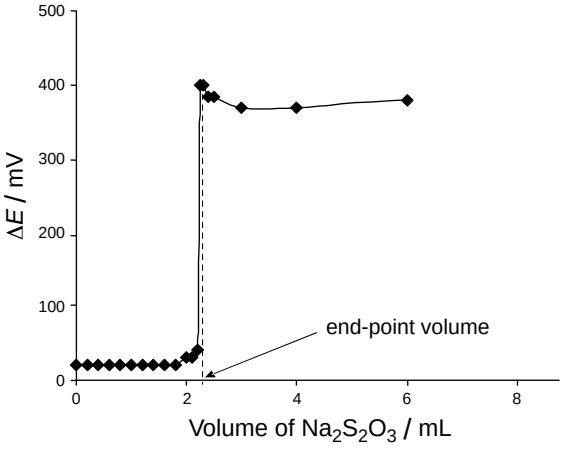

Figure 3. Typical titration curve for a kiwi sample at a current of 0.25 μA.

Results

A titration curve of a kiwi sample, working at a current of 0.25 μA, is shown in Figure 3. As described before, the quantity of AA present in the samples, expressed in mg/100 g, can be calculated from the difference between the total triiodide equivalents generated according to eq 1 and the excess triiodide equivalents titrated with thiosulfate (eq 3), using the following equation

$$\frac{\text{mg AA}}{\text{100g sample}} = \left(\frac{100}{\text{sample mass in g}}\right)\left(\frac{MW_{AA}}{2}\right) \times \left[\left(\text{Vol}_{K_2Cr_2O_7}\text{ in mL}\right)N_{K_2Cr_2O_7} - \left(\text{Vol}_{Na_2S_2O_3}\text{ in mL}\right)N_{Na_2S_2O_3}\right] \quad (4)$$

where MW_{AA} is the molar mass of ascorbic acid, $\text{Vol}_{K_2Cr_2O_7}$ and $N_{K_2Cr_2O_7}$ are the volume (mL) and normality of $K_2Cr_2O_7$ solution added, respectively, and $\text{Vol}_{Na_2S_2O_3}$ and $N_{Na_2S_2O_3}$ are the volume (mL) and normality of the $Na_2S_2O_3$ solution consumed up to the end point, respectively. Examples of the quantities of AA in fruits and vegetables obtained by students are shown in Table 1.

Hazards

Students deal with hazardous compounds, including phosphoric acid, potassium dichromate, and the resulting chromium solution obtained after the titration. Dichromate is a strong oxidant and is harmful if inhaled or in contact with eyes and skin. It has also been identified as a carcinogenic agent. Therefore, students should handle the dichromate solution with care and under supervision. Hazards of phosphoric acid are the result of its corrosive action. Although the stock phosphoric acid solution is not highly concentrated (pH 0.5), precautions should be taken while handling it. Chromium compounds are considered toxic and may cause long-term adverse effects in the environment; therefore, they should be disposed safely. A procedure proposed by Kalbus *(22)* is recommended for the disposal of solutions containing chromium.

Table 1. Ascorbic Acid Content of Fruits and Vegetables Obtained by Students

Sample	mg/100g
Kiwi fruit	113
Tomato	29
Spinach	112
Green pepper	80

Conclusion

The principles of voltammetry are introduced to students by means of a bipotentiometric method to determine vitamin C in fruits and vegetables. As vitamin C is known to play a crucial role in human health, students respond enthusiastically to a lab experiment that allows them to verify a quality marker of the food they usually consume. This experiment has proved to be useful to draw students' attention to electrochemical principles.

[W]Supplemental Material

Instructions for the students and notes for the instructor are available in this issue of *JCE Online*.

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