**Determination of Antioxidant Activity of Vitamin C by Voltammetric Methods †**

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**Abstract:** Voltammetric methods—cyclic (CV) and differential pulse voltammetry (DPV) are considered the most appropriate way to evaluate antioxidant activity of redox active compounds. They provide information about both mechanism and kinetics of electrochemical oxidation of antioxidants as well as their physical and chemical properties such as the redox potential or the number of electrons transferred. These methods are helpful for understanding the mechanisms of oxidation or reduction processes of antioxidant compounds. This work presents the electrochemical properties of vitamin C obtained by both CV and DPV methods.

**Keywords:** L-ascorbic acid; vitamin C; antioxidant activity; cyclic voltammetry; differential pulse voltammetry; electrooxidation

**1. Introduction**

Redox reactions, describing oxidation-reduction via electron transfer, occur commonly in cells of living organisms in order to maintain metabolism and to generate energy. Reactive oxygen (ROS) and nitrogen (RNS) species are produced during normal metabolism and take part in redox signalling that governs cell fate [1]. However, an excessive level of ROS causes oxidative stress and various harmful effects. Thus, the maintenance of cellular redox homeostasis is crucial for healthy living. Subsequently, natural antioxidants derived from foods such as fruits or spices have received a great deal of attention in supporting endogenous antioxidant system of cells [1,2]. One of the food ingredients that plays a key role in supporting antioxidant barrier of the body is L-Ascorbic acid (AA), best known under a common name vitamin C. The reduced form of AA is capable of reacting with strong oxidants (compounds with high value of E°) which can be evaluated by voltammetric methods — cyclic (CV) and differential pulse (DPV) voltammetry. Voltammetric techniques are based on the measurement of the current arising from oxidation or reduction on the electrode surface, following the application of variable potential. CV and DPV are characterized by good sensitivity, thus they are very suitable methods for a wide range of applications and the most effective electro-analytical technique for the study of electroactive species [3]. The objective of the work is to present electrochemical parameters that can be obtained by carrying out CV and DPV measurements of AA in aqueous solution (pH = 7.4).
2. Materials and Methods

2.1. Materials

For the electrochemical studies L-(+)-ascorbic acid (AA) from Sigma Aldrich (Saint Louis, MO, USA) was used. Sodium phosphate buffer prepared using Na₂HPO₄∙12H₂O and NaH₂PO₄∙2H₂O (Sigma Aldrich, Saint Louis, MO, USA) was applied. To clean working electrode and electrochemical cell, H₂SO₄ (POCH, Gliwice, Poland) and potassium permanganate (Sigma Aldrich, Saint Louis, MO, USA) were used. Water, purified using a QPLUS185 system from Millipore (Burlington, MA, USA), was applied.

2.2. Methods

Voltammetric measurements were carried out using a Gamry Reference 600 potentiostat (Gamry Instruments, Inc., Warminster, PA, USA) and were performed in a three-electrodes system consisting of a glassy carbon electrode (GC, 1.6 mm in diameter), platinum wire and the 3 M KCl Ag|AgCl (type R-10/S, Hydromet s.c., Gliwice, Poland), which were the working (WE), the counter (CE) and the reference (RE) electrode, respectively. Different concentrations of AA (2, 3, 4, 5, 6 mM) were prepared by dissolving it in 1 M sodium phosphate buffer as the supporting electrolyte (pH = 7.4 ± 0.1). Prior to the measurements, the solution was deoxygenize by 3 min percolation with high purity argon and the surface of WE was carefully polished using alumina suspension (MicroPolish Alumina, 0.05 μm particles, Buehler, Lake Bluff, IL, USA) on microcloth pads (MF-1040, BASi, West Lafayette, IN, USA) and then rinsed with distilled water and methanol. The RE was stored in 3 M KCl and rinsed with water prior to use. All cyclic (CV) and differential pulse (DPV) voltammograms were performed at positive potentials, in the range -0.3 to +0.8 V. The number of cycles was set at 4 in CV. To assess the impact of scan rate on the oxidation process of tested samples, the following rates were selected: 10, 20, 30, 50 and 100 mV/s. In the case of DPV, the settings were: pulse size 50 mV, pulse time 0.1 s and sample period 0.5 s. All experiments were performed at 25 °C. Temperature was controlled using Ultra Thermostat AD 07R-20-A12E model (PolyScience, Niles, IL, USA). Three independent measurements for each sample were performed. Voltammograms were analyzed by SigmaPlot 13.0 software (Systat Software Inc., London, UK).

3. Results

Electrochemical oxidation of AA at the GC electrode was studied by cyclic and differential pulse voltammetry. Selected CV and DPV voltammograms for the electrooxidation of vitamin C and the supporting electrolyte are shown in Figure 1A.

![Figure 1](image-url)

**Figure 1.** (A) CV—cyclic voltammogram DPV—differential pulse voltammogram; c = 4 mM, potential scan rate (v) 100 mV/s. (B) CVs, c = 4 mM at various v; 100 mV/s, 50 mV/s, 30 mV/s, 20 mV/s, 10 mV/s of the supporting electrolyte. (C) The dependence of the anodic peak current (Iₚ,a) on the square root of v, c = 4 mM. (D) The dependence of the anodic peak potential (Eₚ,a) on the v for various concentrations: 2 mM, 3 mM, 4 mM, 5 mM, 6 mM. (E) CVs for various concentrations, v = 10
mV/s. (F) The dependence of \( I_{p,a} \) for various concentrations, \( v = 10 \text{ mV/s} \); electrooxidation of vitamin C at GC electrode, solutions prepared in 1 M sodium phosphate buffer.

The supporting electrolyte (1 M sodium phosphate buffer) shows no characteristic peaks, thus the observed peaks are the results of oxidation of AA. The values of the anodic peak potential and current are well determined for electrode reaction. The received voltammograms (Figure 1A) show that AA is irreversibly oxidized in one step, where two electrons are changing. The potential anodic peak \( (E_{p,a}) \), as determined by CV is 0.34 V \((\text{vs.} \text{RE})\). This is somewhat variable with concentration (Figure 1D), thus suggesting that potential found in this way may not be correlated with antioxidant activity measured by biochemical and biological tests. The influence of potential polarisation rate on the electrooxidation of AA was studied by CV with scan rates of 10–100 mV/s (Figure 1B). The analysis of linear dependence between \( I_{p,a} \) on \( v^{1/2} \) or \( \ln(I_{p,a}) \) on \( \ln(v) \) allows to define whether a reaction is controlled by adsorption or diffusion processes [4].

The influence of AA concentration on the reaction on the GC electrode was investigated in the concentration range from 2 mM to 6 mM. CV and the dependence of \( I_{p,a} \) on the concentration is presented in Figures 1E and 1F. This dependence is described by linear regression in over the whole concentration range of AA. The linear dependence of \( I_{p,a} \) on concentration of the depolarizer (Figure 1F) is maintained also at other scan rates.

4. Discussion

Reactions on the surface of the electrode are characterized by the dependence of the current on the electrode potential. Potential of the DPV anodic peak is shifted relative to CV (Figure 1A). This shift results from the difference in measured current in both techniques. DPV as a pulsed technique is highly sensitive, because the charging current (non faradaic process) is minimized. Thus, the height of the peak current in DPV is directly proportional to the concentration of redox active species in solution, while in CV both faradaic and non-faradaic currents are measured. Considering studied potential scan rate ranging from 0.01 to 0.1 V/s, the anodic peak current depends linearly on the square root of the scan rate. It is described by the following Equation (1) (4 mM AA, Figure 1C):

\[
I_{p,a} = 160.4 \, (v)^{1/2} + 14.9 \quad r^2 = 0.9944
\]

This equation may suggest whether the electrode reaction is diffusion or adsorption-controlled. Moreover, the dependence of \( \ln(I_{p,a}) \) on \( \ln(v) \) is characterized by linear regression (Equation (2)):

\[
\ln(I_{p,a}) = 0.3198 \ln(v) + 4.9 \quad r^2 = 0.9803
\]

The slope of the fit reaches 0.3198, which suggests that the process is controlled by diffusion. Diffusion-controlled electrode processes are characterized by value of the slope close to 0.5. However, processes, which are controlled by adsorption are described by a slope close to 1.0 [5–8]. The same results are observed for other investigated concentrations of vitamin C. For a reversible reaction, the oxidation peak potential is independent of \( v \). It may be considered that the heterogeneous electron transfer at peak is irreversible, thus determination of values of the electron transfer coefficient \( (\beta_{\text{nt}}) \) for the reaction is possible [5]. The following Equation (3) is valid for a totally irreversible diffusion-controlled process.

\[
E_{p,a} = \frac{RT}{2nF} \ln v + \text{const}
\]

where: \( E_{p,a} \) — anodic peak potential (V), \( R \) — gas constant \((8.314 \text{ JK}^{-1}\text{mol}^{-1})\), \( F \) — Faraday constant \((96487 \text{ Cmol}^{-1})\), \( T \) — temperature (K), \( \beta_{\text{nt}} \) — anodic transfer coefficient, \( v \) — scan rate \((\text{Vs}^{-1})\).

Based on the dependence of \( E_{p,a} \) on \( \ln(v) \), described by following Equation (4), \( \beta_{\text{nt}} \) is equal to 0.65 for electrooxidation of 3 mM AA.

\[
E_{p,a} = 0.0199 \cdot \ln(v) + 0.4019 \quad r^2 = 0.9938
\]
Analysis of the CVs received allows to calculate the *Tafel slope* ($b$ – in equation 5) as information on the rate-determining step. Equation (5) describes an irreversible process, which is controlled by diffusion [8,9].

\[
E_{pa} = b/2\ln(v) + \text{const}
\]

(5)

The slope above dependence is equal 19.9 mV, thus *Tafel slope* was found to be 39.8 mV in this work.

5. Conclusions

Vitamin C is considered as a compound with strong antioxidant activity. In this study, the electrochemical behavior of AA at GC electrode was investigated. It was observed, that this compound is irreversibly oxidized in one electrochemical step with the exchange of two electrons. Based on cyclic voltammetry technique, electrochemical parameters (the anodic transfer coefficient, the Tafel slope) were calculated. These values help to understand the kinetics of underlying reactions of redox active compounds. Voltammetric studies may provide information on electrochemical oxidation mechanisms of other ascorbate compounds in aqueous media.

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**Conflicts of Interest:** The authors declare no conflict of interest.

**References**


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