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Electrochemical Determination of Eplerenone in Pharmaceutical Formulations using Cyclic and Square-Wave Voltammetry

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A voltammetric method was developed to determine eplerenone (EPL), a selective aldosterone antagonist, in tablet formulations. Electrochemical tests showed that EPL undergoes irreversible, diffusion-controlled oxidation at a glassy carbon electrode. Both cyclic voltammetry (CV) and square-wave voltammetry (SWV) were used, and the conditions were optimized to give stable and reproducible signals. A linear relation was obtained between peak current and EPL concentration over 10–180 μg mL^{-1}, with detection and quantification limits of 7.18 and 21.8 μg mL^{-1}. The method was also tested on commercial tablets and produced accurate results, confirming its use for routine analysis. Along with good sensitivity, it is fast, inexpensive, and does not require complex sample preparation, making it a practical tool for quality control of EPL in the pharmaceutical industry.

Keywords: eplerenone, cyclic voltammetry, square-wave voltammetry, pharmaceutical quality control, analytical method

Introduction

Cardiovascular diseases (CVDs) remain the main cause of death worldwide and continue to pose a major challenge for public health. In the United States, heart disease is responsible for roughly one in four deaths each year, while in Iraq almost one-third of all deaths are linked to CVDs, according to the World Health Organization [1,2]. Although hereditary factors play a role, most cases are related to lifestyle choices such as diet, lack of physical activity, smoking, substance use, and long-term stress [3–5]. This makes prevention and effective treatment essential, as well as the need for analytical methods that allow accurate monitoring of drug levels and treatment outcomes.

Electrochemical techniques, especially voltammetry, have gained attention in recent years as practical tools for drug analysis [6,7]. These methods are valued for their high sensitivity, short analysis time, and ability to provide information on oxidation–reduction behavior in complex biological systems [8]. Unlike more expensive or labor-intensive methods, voltammetry can be applied directly to pharmaceutical formulations or biological samples, giving both quantitative data and mechanistic insights [9].Eplerenone (EPL) is a selective aldosterone antagonist widely used in the treatment of cardiovascular disease. It is a derivative of spironolactone with structural changes that include a 9α,11α-epoxy bridge and substitution of the 17α-thioacetyl group with a carbomethoxy substituent (Figure 1) [10–13]. These modifications improve its pharmacological profile, but the electrochemical behavior of EPL has not been fully investigated.

The present study focuses on the voltammetric characterization of EPL at a glassy carbon electrode (GCE). By examining its oxidation processes under optimized conditions, we aim to clarify the redox mechanism and explore how these findings can be applied to pharmaceutical quality control and drug monitoring. Such work not only adds to the understanding of EPL itself but also highlights the broader usefulness of voltammetry in pharmaceutical analysis and cardiovascular therapy [14].

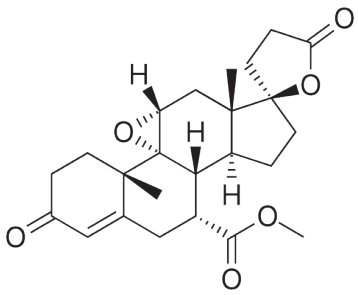

Figure 1. Chemical structure of eplerenone ($C_{24}H_{30}O_6$).

Experimental part

Chemicals and solutions

Eplerenone (purity ≥99%) was obtained from Nanjing Duly Biotechnology Co., Ltd. A stock solution of 150 μg·mL^{-1} was freshly prepared each day in 0.1 M Britton–Robinson buffer (BRB). The BRB was prepared by mixing equal volumes of 0.1 M phosphoric acid (Dow Chemical Co.), acetic acid (Eastman Chemical

Co.), and boric acid (BASF), and the pH was adjusted to a range of 2.0–12.9. All reagents were of analytical grade, and solutions were prepared using Milli-Q deionized water. Unless otherwise specified, 0.1 M BRB was employed as the supporting electrolyte for electrochemical experiments.

A commercial pharmaceutical formulation (EPLIMAX, Arifrye, Sakarya, Turkey), labeled to contain 25 mg of EPL per tablet, was purchased from a local market. For sample preparation, tablets were accurately weighed, finely powdered, and an amount equivalent to one tablet was dispersed in BRB. The suspension was subjected to ultrasonic treatment for 10 min, filtered, and quantitatively transferred to a 50 mL volumetric flask. Appropriate dilutions with BRB were carried out to obtain concentrations within the calibration range.

Apparatus and procedure

Electrochemical experiments were carried out with a DY-2000 potentiostat/galvanostat (USA) using a standard three-electrode cell (50 mL). The glassy carbon electrode (GCE, 1 mm diameter) was used as the working electrode, a platinum wire as the counter electrode, and Ag/AgCl (3.0 M KCl) as the reference.

Before each run, the GCE was polished with diamond suspension and alumina slurry, rinsed with deionized water, and cycled until a stable background current was obtained. Conditioning was done by cyclic sweeping between −0.2 and +0.2 V (vs. Ag/AgCl) in 0.1 M BRB at 0.001 V·s^{-1} until reproducible voltammograms appeared.

Cyclic voltammetry (CV) was performed in the potential range of −1.1 to +0.1 V with scan rates between 0.001 and 0.05 V·s^{-1}. Square-wave voltammetry (SWV) was run under optimized settings: initial potential −0.58 V, final potential −0.90 V, step potential 0.04 V, modulation amplitude 0.025 V, and frequency 2 Hz.

Electrochemical procedure

The redox behavior of EPL was studied by cyclic voltammetry (CV) and square-wave voltammetry (SWV) to clarify its oxidation mechanism and optimize the experimental conditions. All measurements were repeated to ensure reproducibility. The influence of pH, scan rate, and temperature on electrode response was systematically examined. In addition, the effect of different supporting electrolytes on the oxidation current of EPL (150 μg·mL^{-1}) was tested in 0.1 M methanol, Britton–Robinson buffer (BRB), acetate buffer, and phosphate buffer.

Results and Discussion

Influence of supporting electrolyte

Supporting electrolyte plays a critical role in defining the electrochemical response of eplerenone (EPL). Cyclic voltammograms were recorded for 150 μg·mL^{-1} EPL in 0.1 M methanol, Britton–Robinson buffer (BRB), acetate buffer, and phosphate buffer. BRB produced the highest and most stable oxidation peak current, so it was chosen as the supporting electrolyte for all further experiments (Figure 2).

Optimization of experimental parameters

The electrochemical oxidation of EPL at the glassy carbon electrode (GCE) was examined by cyclic voltammetry. Voltammograms were recorded at scan rates of 0.001–0.05 V·s^{-1} in 0.1 M Britton–Robinson buffer (Figure 3). The anodic peak current increased with the square root of the scan rate, showing that the process is mainly diffusion-controlled.

Kinetic analysis gave a slope of 0.1338 for log(Ip) versus log(v), much lower than the theoretical 0.5 expected for a fully reversible diffusion process. Along with the positive shift of peak potential at higher scan rates, this indicates that the oxidation of EPL is irreversible. Using Laviron's model for irreversible systems [15,16], the slope parameter was found to be b = 0.0294, corresponding to about 1.75 electrons transferred. These results suggest that EPL undergoes an irreversible two-electron oxidation, in agreement with earlier findings for similar systems [17].

Effect of pH

The effect of pH on the anodic response of eplerenone (150 μg·mL^{-1}) was studied in Britton–Robinson buffer over the range pH 2.0–12.9 at a fixed scan rate. The oxidation peak potential (Ep) shifted to less positive values as pH increased, showing that proton transfer is linked to the electron-transfer step.

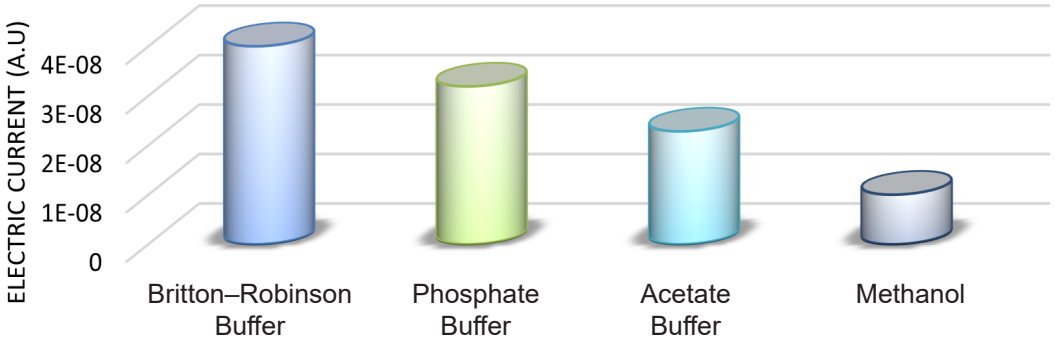

Figure 2. Effect of electrolyte type on the electrochemical behavior of EPL at the GCE.

A strong linear correlation was obtained, described by Ep (V) = –0.056 pH – 0.3426 (R^2 = 0.9956). The slope of –0.056 V/pH is very close to the theoretical Nernst value of –0.059 V, indicating that the number of protons involved is equal to the number of electrons transferred. Similar proton–electron coupling has been reported in other electrochemical sensor studies [18,19].

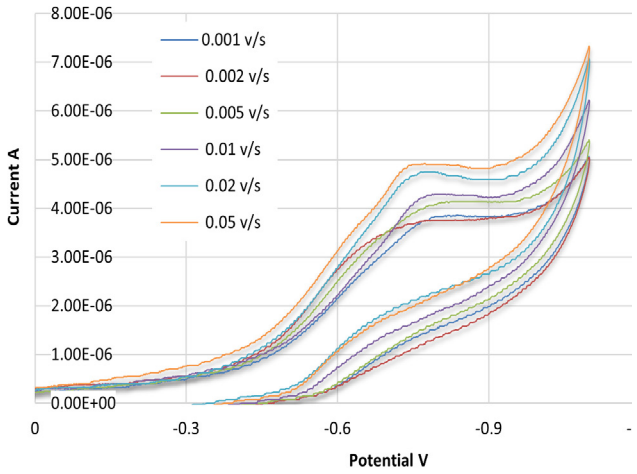

Figure 3. Cyclic voltammograms of EPL (150 μg·mL^{-1}) in BRB at different scan rates.

Effect of temperature

The influence of temperature on the electrochemical response of eplerenone (150 μg·mL^{-1} in 0.1 M Britton–Robinson buffer, scan rate 0.05 V s^{-1}) was investigated over the temperature range of 5–40 °C. The anodic current increased progressively with temperature, reaching a maximum at around 35–40 °C, indicating that higher temperatures favor the electron-transfer kinetics at the glassy carbon electrode.

Thermodynamic parameters were evaluated using the Eyring approach. A linear plot of log(Ipa) versus 1/T gave a slope of –205.24, from which activation and thermodynamic values were calculated. The apparent activation energy (Ea) was 3.93 kJ·mol^{-1}, and the enthalpy change (ΔH) was 0.11 kJ·mol^{-1}. The entropy change (ΔS) was negative (–178.4 J·mol^{-1}·K^{-1}), indicating that oxidation creates a more ordered interfacial system. The Gibbs free energy (ΔG) was positive (55.79 kJ·mol^{-1}), showing that the electrode process is non-spontaneous under the studied conditions. Similar findings have been described in other electrochemical systems, including electrode degradation and oxygen reduction [20].

Electrochemical behavior of eplerenone

The redox behavior of eplerenone at a glassy carbon electrode (GCE) was evaluated by cyclic voltammetry (CV) and square-wave voltammetry (SWV) in 0.1 M Britton–Robinson buffer. At a concentration of 150 μg·mL^{-1}, the SWV scans performed between –0.2 and +1.9 V at 0.05 V·s^{-1} revealed a well-defined anodic response (Figure 4). No cathodic counterpart was detected on the reverse scan, confirming the irreversible nature of the oxidation process.

In CV measurements, eplerenone produced a clear anodic peak at approximately –1.26 V with a peak current of 1.51×10^{-5} A. Control experiments using the blank supporting electrolyte showed no detectable peaks, while the presence of eplerenone consistently generated a sharp oxidative signal. These results demonstrate that the electrode process arises exclusively from the analyte and confirm that the oxidation of eplerenone at the GCE is both irreversible and highly reproducible under the optimized conditions.

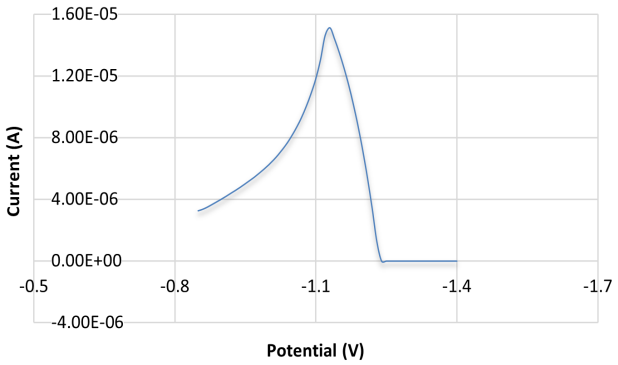

Figure 4. Square-wave voltammogram of 150 μg·mL^{-1} eplerenone recorded at a glassy carbon electrode in 0.1 M Britton–Robinson buffer under optimized conditions.

Interference study

The selectivity of the glassy carbon electrode (GCE) for eplerenone oxidation was tested in the presence of common excipients used in 25 mg tablet formulations, including lactose monohydrate, magnesium stearate, and microcrystalline cellulose. Square-wave voltammetry (SWV) measurements (Figure 5) showed that even a tenfold excess of these substances did not significantly affect the anodic peak current of eplerenone. This confirms the good selectivity of the method, consistent with other voltammetric studies reporting minimal interference from excipients [18,19].

Calibration graph for eplerenone

To validate the analytical method for the detection and quantification of eplerenone, square-wave voltammetry (SWV) was employed under optimized conditions. Oxidation peak currents were recorded at the glassy carbon electrode (GCE) for eplerenone concentrations ranging from 10 to 180 μg·mL^{-1} in 0.1 M Britton–Robinson buffer. As the analyte concentration increased, the anodic peak currents rose proportionally, demonstrating excellent linearity.

The regression equation describing the calibration curve was: Ipa (A) = 0.0003 [C, μg·mL^{-1}] + 1.3025 with a correlation coefficient R^2 = 0.9988. Statistical parameters derived from five replicate determinations are summarized in Table 1.

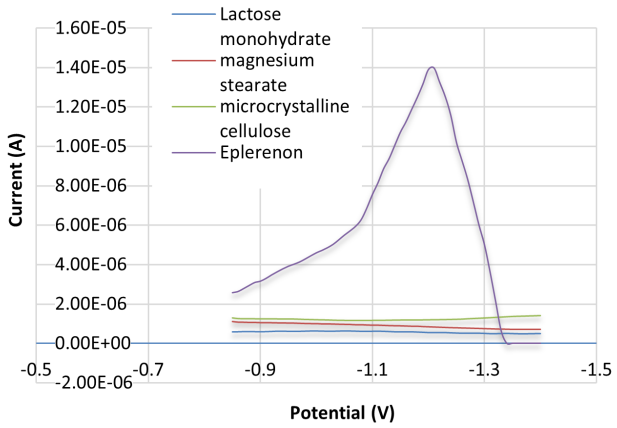

Figure 5. Effect of common pharmaceutical excipients on the voltammetric response of eplerenone using the SWV technique.

Table 1. Analytical parameters of the calibration plot for eplerenone using SWV at the glassy carbon electrode.

Parameter	Value
Linearity range (μg·mL^{-1})	10 – 180
Slope (A·μg^{-1}·mL)	0.0003
Intercept (A)	1.3025
Correlation coefficient (R^2)	0.9988
LOD (μg·mL^{-1})	7.18
LOQ (μg·mL^{-1})	21.8
SD (A)	1.58×10^{-6}
RSD (%)	4.13
Percentage error (%)	0.366

Analytical application of the method

The applicability of the voltammetric method was tested by recovery experiments on pharmaceutical samples containing eplerenone at different concentrations. Known amounts of the drug were added to pre-analyzed formulations, and recoveries were calculated using the calibration curve.

As shown in Tables 2, the results were in excellent agreement with the labeled content of the products. Standard addition confirmed a linear rise in anodic peak current with increasing eplerenone concentration, supporting the accuracy of the method. High recovery values together with low relative standard deviations demonstrate that both cyclic voltammetry (CV) and square-wave voltammetry (SWV) are reliable, reproducible, and suitable for routine analysis of eplerenone in bulk drug and finished formulations.

Conclusion

In this work, the electrochemical behavior of eplerenone was investigated at a glassy carbon electrode using cyclic voltammetry and square-wave voltammetry. The oxidation of the drug was shown to be an irreversible process involving two electrons and coupled proton transfer. Britton–Robinson buffer (0.1 M) provided the best medium for the electrochemical response and was used as the supporting electrolyte in all subsequent experiments.

The influence of different parameters such as scan rate, pH, and temperature was examined in detail, allowing characterization of the electrode process and calculation of thermodynamic parameters. Square-wave voltammetry enabled the construction of a reliable calibration curve over the concentration range 10–180 μg·mL^{-1}, with detection and quantification limits of 7.18 and 21.8 μg·mL^{-1}, respectively. Recovery tests performed on commercial tablet formulations gave results in good agreement with the labeled content, with low relative standard deviations.

Obtained results confirm that the proposed voltammetric procedure can be used for the determination of eplerenone in both bulk material and pharmaceutical dosage forms. The method is simple to perform, does not require complicated sample preparation, and provides acceptable levels of sensitivity, selectivity, and precision for routine laboratory analysis.

Table 2. Determination of eplerenone in bulk and pharmaceutical formulations using cyclic voltammetry (CV) and square-wave voltammetry (SWV).

Sample type	Nominal (mg)	Found (mg)	Recovery (%)	RSD (%)	Method
Bulk	16.00	15.92	100.50	0.500	CV
	36.00	35.78	100.60	0.176	
	85.00	84.94	99.98	0.0597	
Pharmaceutical formulation	25.00	24.85	100.60	0.210	
	50.00	50.01	99.98	0.121	
	75.00	74.89	100.15	0.110	
Bulk	16.00	15.95	100.30	1.470	SWV
	36.00	35.97	100.08	0.362	
	85.00	85.04	99.95	0.526	
Pharmaceutical formulation	25.00	24.81	100.76	0.951	
	50.00	49.97	100.60	0.116	
	75.00	75.03	99.96	0.0912	

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