Electrochemical Determination of Testosterone in Bulk and Pharmaceutical Formulations by Cyclic Voltammetry

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Testosterone (TST), a widely used anabolic steroid, requires sensitive and selective analytical approaches for reliable determination in pharmaceutical formulations and environmental matrices. In this work, its electrochemical properties were examined using cyclic voltammetry under optimized conditions (pH 12.7, 40 °C, scan rate 50 mV·s^{-1}). TST exhibited an irreversible, diffusion-controlled oxidation process. A linear calibration curve was obtained within the concentration range of 3.47×10^{-5} – 3.47×10^{-4} M ($R^2 = 0.999$). The method achieved a detection limit (LOD) of 2.08×10^{-5} M and a quantification limit (LOQ) of 6.30×10^{-5} M. Thermodynamic parameters were calculated as $\Delta H = 2.59$ kJ·mol^{-1}, $\Delta S = 392.22$ J·K^{-1}·mol^{-1}, and $\Delta G = -120.22$ kJ·mol^{-1}, confirming the spontaneity of the redox process. The electrode's electroactive surface area was 0.0054 cm^2. High precision was demonstrated, with relative standard deviations of less than 0.5%. The method was successfully applied to the quantification of testosterone in bulk and pharmaceutical formulations, with recoveries consistent with certified values.

Keywords: testosterone, cyclic voltammetry, electrochemical determination, pharmaceutical analysis, irreversible redox process

Introduction

The quantitative determination of testosterone (TST, 17β-hydroxyandrost-4-en-3-one) represents a critical objective in both pharmaceutical and clinical analytical chemistry. Testosterone is the principal endogenous androgenic–anabolic steroid in humans, synthesized primarily in the testes of males and the ovaries of females, with minor production in the adrenal glands [1]. It plays an essential physiological role in reproductive function, the development of secondary sexual characteristics, and the maintenance of skeletal muscle and bone mass. Abnormally low testosterone levels have been associated with a range of health disorders, including osteoporosis, obesity, mood alterations, and sexual dysfunction [2]. Consequently, accurate, rapid, and reliable analytical methods for testosterone quantification in bulk materials and pharmaceutical preparations are essential for ensuring patient safety and effective quality control.

A variety of analytical methodologies have been developed for the determination of testosterone. Traditional spectroscopic and chromatographic techniques, such as UV–visible spectrophotometry [3] and high-performance liquid chromatography (HPLC) [4], offer excellent accuracy and selectivity but incur high operational costs, lengthy procedures, and substantial solvent consumption. Immunoassays and biosensors have been introduced as highly sensitive alternatives; however, their performance may be compromised by matrix effects, cross-reactivity, and high reagent expenses [5,6]. These drawbacks have stimulated growing interest in greener, faster, and more cost-efficient analytical strategies.

Electrochemical methods have emerged as promising alternatives due to their inherent advantages of high sensitivity, low cost, minimal reagent use, and rapid analysis [7]. Among these, voltammetric techniques, such as cyclic, linear sweep, square-wave, and stripping voltammetry, enable both mechanistic elucidation of redox processes and quantitative analysis of analytes at trace levels [8,9]. Furthermore, modifications to electrode surfaces have enhanced the selectivity and sensitivity of electrochemical systems for detecting biologically active compounds [10]. Cyclic voltammetry (CV), in particular, offers unique versatility by simultaneously providing insight into redox behavior and enabling reliable quantification [11,12].

The present study aims to establish cyclic voltammetry as a sensitive, selective, and sustainable approach for the determination of testosterone in bulk and pharmaceutical formulations. Through systematic optimization of experimental parameters and validation of analytical performance, this work contributes both mechanistic understanding and practical methodology for pharmaceutical quality control applications.

Materials and methods

Chemicals and reagents

Testosterone (TST, ≥ 99% purity) was obtained from Darmstadt, Germany. Ethanol (≥ 99%) was purchased

from Salius Pharma (India). Sodium hydroxide (NaOH, ≥99%) was supplied by Fluka (Switzerland), and potassium hexacyanoferrate(III) ($K_3[Fe(CN)_6]$) was purchased from Sigma-Aldrich (USA). A stock solution of testosterone (3.0×10^{-3} mol·L^{-1}) was prepared by dissolving the appropriate amount in ethanol. Working solutions were freshly prepared by diluting the stock solution with deionized water. Sodium hydroxide solutions were used as supporting electrolytes and prepared daily to a final volume of 25 mL.

Pharmaceutical formulations containing testosterone were finely powdered, and an accurately weighed portion was dissolved in ethanol, sonicated for 10 min, and filtered through a 0.45 μm membrane filter. Suitable aliquots were then diluted with deionized water to obtain the required concentrations for analysis. All solutions were equilibrated to room temperature prior to use.

Instrumentation

Electrochemical measurements were carried out using a DY2100 series potentiostat. A conventional three-electrode configuration was employed, consisting of a glassy carbon electrode (GCE, 3 mm diameter) as the working electrode, a platinum wire as the auxiliary electrode, and an Ag/AgCl (saturated KCl) electrode as the reference. The pH of all solutions was adjusted and monitored with a digital pH meter equipped with a combined glass electrode.

Electrochemical measurements

The electrochemical behavior of testosterone was studied by cyclic voltammetry (CV). Measurements were performed in the potential range of –0.2 to +1.2 V (vs. Ag/AgCl) under optimized conditions: pH 12.7, scan rate of 50 mV·s^{-1}, and temperature of 40 °C. Before each experiment, the glassy carbon electrode was polished with alumina slurry, rinsed with deionized water, and dried at room temperature. Background currents were corrected using the blank supporting electrolyte. All experiments were carried out in triplicate to ensure reproducibility.

Results and discussion

Electrode active surface area

The electroactive surface area of the glassy carbon electrode (GCE) was evaluated using cyclic voltammetry of 5 mM potassium ferricyanide ($K_3[Fe(CN)_6]$) in 0.1 M KCl at different scan rates (Figure 1). Cyclic voltammograms were recorded in the potential window from −0.2 to +0.8 V (vs Ag/AgCl), and the corresponding anodic peak currents (I_{pa}) were analyzed according to the Randles–Ševčík equation for a reversible diffusion-controlled process [13]:

$$I_{pa} = 0.4463(F^3/RT)^{1/2} n^{3/2} AD^{1/2} v^{1/2}, \text{ (Eq. 1)}$$

where A is the electrode area (cm²), D the diffusion coefficient (cm²·s^{-1}), n the number of electrons transferred, v the scan rate (V·s^{-1}), F the Faraday constant, R the gas constant, and T the absolute temperature.

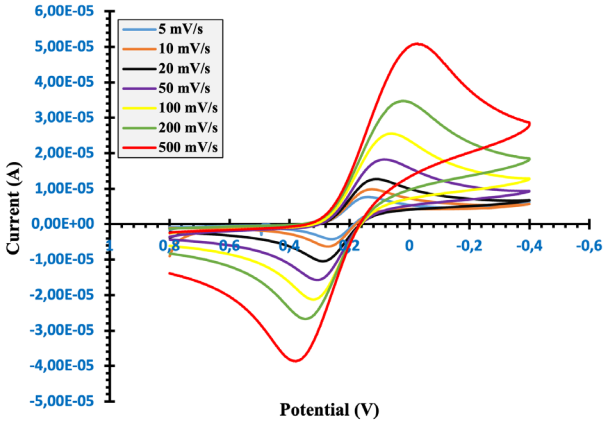

Figure 1. Cyclic voltammograms of 5 mM $K_3[Fe(CN)_6]$ at different scan rates.

A linear relationship between the anodic peak current and the square root of the scan rate ($v^{1/2}$) was observed (see Figure S1 in the *Supplementary Materials*), confirming that the redox process of ferricyanide at the GCE is diffusion-controlled. Based on the slope of this plot and the known diffusion coefficient for $[Fe(CN)_6]^{3-}$ (7.6×10^{-6} cm²·s^{-1}), the electroactive surface area of the electrode was calculated as 0.0054 cm². These results indicate good electrode activity and reproducibility of the surface, providing a reliable basis for subsequent electrochemical measurements of testosterone

Effect of scan rate

The influence of scan rate on the electrochemical behavior of testosterone was examined in 0.5 M NaOH (pH 12.7) at the glassy carbon electrode (GCE) using cyclic voltammetry. Voltammograms were recorded over the range 5–200 mV·s^{-1} (Figure 2). The oxidation peak current increased proportionally with scan rate, and the most reproducible and well-defined responses were obtained at 50 mV·s^{-1}, which was therefore selected as the optimal condition for subsequent analyses.

A linear relationship between the anodic peak current density (J_{pa}) and the square root of the scan rate ($v^{1/2}$) was observed, consistent with the Randles–Sevcik equation for a diffusion-controlled process (Equation 1). In addition, the logarithmic plot of current density versus scan rate (Figure S2, *Supplementary Materials*) followed the regression equation:

$$\log J = 0.5345 \log v + 17.102 \ (R^2 = 0.9977) \text{ (Eq. 2)}$$

The slope of 0.5345 is very close to the theoretical value of 0.5 for diffusion control, while adsorption-controlled processes typically approach unity [14,15]. These results confirm that testosterone oxidation at the GCE is governed primarily by diffusion.

The irreversible nature of the process was further verified by examining the dependence of the peak potential (E_p) on the scan rate (Figure S3, *Supplementary Materials*). According to Laviron's equation for irreversible electrode reactions [16]:

$E_p = E^0 + [2.303RT/\alpha nF] \log (RTk^0/\alpha nF) + [RT/\alpha nF] \log v$ (Eq. 3)

From the slope and intercept of the E_p–log v plot, the electron transfer coefficient (α), standard heterogeneous rate constant (k^0), and formal redox potential (E^0) were calculated. The obtained values were: E^0 = 0.744 V, α = 0.426, k^0 = 0.447 s^{-1}. The number of electrons involved was estimated as n ≈ 2, consistent with a two-electron irreversible oxidation pathway for testosterone. The diffusion coefficient (D_0) was also determined as 3.15 × 10^{-4} $cm^2 \cdot s^{-1}$ at the optimal scan rate of 50 $mV \cdot s^{-1}$.

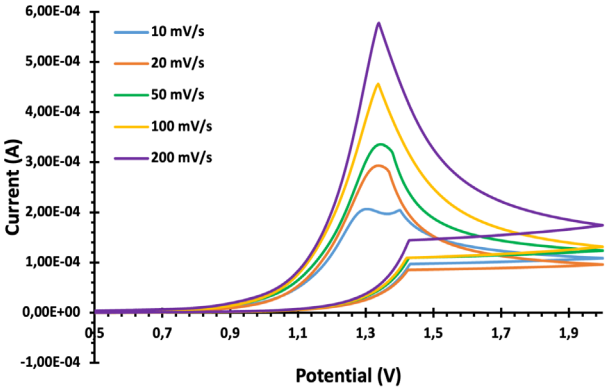

Figure 2. Cyclic voltammograms of testosterone at the GCE at different scan rates.

Effect of pH

The influence of pH on the electrochemical oxidation of testosterone was investigated in the pH range of 7–14 using sodium hydroxide (0.1–0.5 M) as the supporting electrolyte. A clear dependence of both the anodic peak current and the peak potential on pH was observed (Figure 3). At pH values below 7, the solutions became turbid, likely due to the limited solubility of testosterone under acidic and near-neutral conditions, which restricted electron transfer at the electrode surface.

With increasing alkalinity, the anodic peak current gradually increased, reaching a maximum at pH 12.7. Under strongly alkaline conditions, testosterone exhibits higher solubility and greater electroactivity, resulting in more efficient oxidation at the glassy carbon electrode (GCE).

The variation of peak potential (E_p) with pH followed a linear trend consistent with a Nernst-type relationship characteristic of diffusion-controlled electrochemical processes [17]:

$E_p = -0.05915 (p\alpha/n)\ pH$ (Eq. 4)

The calculated slope (pα/n) was 0.556, which is significantly lower than unity, suggesting that proton transfer is not directly involved in the rate-determining step of testosterone oxidation. This indicates that the process is primarily diffusion-controlled, with only partial proton participation.

At pH values above 12, the anodic peak currents increased sharply, confirming that alkaline conditions strongly facilitate the electro-oxidation of testosterone. Based on these results, pH 12.7 was selected as the optimal condition for further experiments (see Figure S4, *Supplementary Materials*).

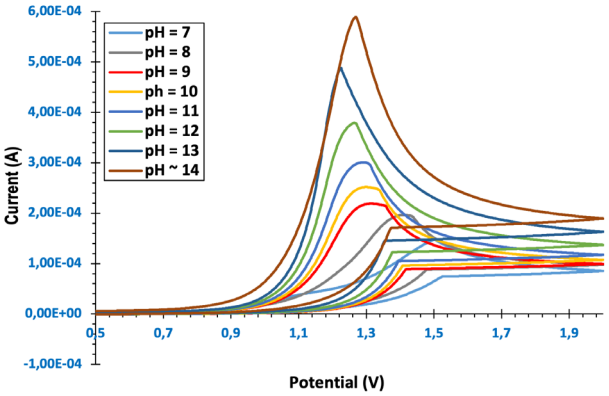

Figure 3. Cyclic voltammograms of testosterone at the GCE at different pH values.

Effect of temperature

The effect of temperature on the electrochemical oxidation of testosterone at the GCE was studied in the range of 10–40 °C at a scan rate of 50 $mV \cdot s^{-1}$ (Figure S5). A gradual increase in the anodic peak current with increasing temperature was observed, indicating that higher temperatures facilitate electron-transfer kinetics. The most favorable electrochemical response was obtained at 40 °C, which was therefore selected as the working temperature for subsequent measurements.

Thermodynamic parameters were evaluated from the linear relationship between log(J/T) and 1/T (Figure S6). From the slope of this plot, the enthalpy change (ΔH) was calculated as 2.59 $kJ \cdot mol^{-1}$, while the intercept yielded an entropy change (ΔS) of 392.35 $J \cdot K^{-1} \cdot mol^{-1}$. The positive ΔH value confirms that testosterone oxidation is an endothermic process, while the positive ΔS indicates an increase in disorder as the system proceeds from reactants to products [18].

The Gibbs free energy change (ΔG) was calculated according to Equation (5):

$\Delta G = \Delta H - T\Delta S$ (Eq. 5)

At 298 K, the ΔG value was −120.22 $kJ \cdot mol^{-1}$, confirming that the electrochemical oxidation of testosterone at the GCE is a spontaneous process under the studied conditions.

Calibration curve

The quantitative determination of testosterone was performed under optimized experimental conditions (pH 12.7, scan rate 50 $mV \cdot s^{-1}$, temperature 40 °C). Cyclic voltammetry measurements at increasing testosterone concentrations produced a proportional rise in anodic peak current density (Figure S7).

A linear calibration curve was established over the concentration range of 3.47 × 10^{-5} – 3.47 × 10^{-4} M (Figure S8, Figure S9). The regression equation was:

$$J\ (\mu A) = 0.1273\ [Conc.] + 124.58 \quad (R^2 = 0.9973) \qquad \text{(Eq. 7)}$$

The high correlation coefficient confirmed excellent linearity of the method. The limit of detection (LOD) and limit of quantification (LOQ) were determined according to the standard formulas $LOD = 3.3\sigma/m$ and $LOQ = 10\sigma/m$, where σ is the standard deviation of the blank and m is the slope of the calibration curve. The calculated values were 2.08×10^{-5} M (LOD) and 6.30×10^{-5} M (LOQ).

The main analytical performance parameters are summarized in Table 1. The low relative standard deviation (RSD = 5.18×10^{-3}) demonstrated high precision, while the narrow linearity range and low detection limits confirmed the suitability of the method for sensitive electrochemical determination of testosterone in pharmaceutical formulations.

Table 1. Analytical performance characteristics of the proposed cyclic voltammetric method for testosterone determination at GCE.

Parameter	Value
Linear range (M)	3.47×10^{-5} – 3.47×10^{-4}
Slope (μA·M^{-1})	47.138
Intercept (μA)	124.58
Correlation coefficient (R^2)	0.9973
LOD (M)	2.08×10^{-5}
LOQ (M)	6.30×10^{-5}
SD (μA)	2.97
RSD (%)	5.18×10^{-3}

Interference studies

To evaluate the selectivity of the proposed method, the influence of common excipients and potentially interfering substances on the electrochemical response of testosterone was examined. The test was carried out in 0.5 M NaOH solution at pH 12.7 and 40 °C, using a testosterone concentration of 500 ppm and a scan rate of 50 mV·s^{-1}. Typical excipients present in pharmaceutical formulations, including lactose, magnesium stearate, and starch, were added individually to the supporting electrolyte (Figure 4).

The results indicated that the oxidation peak current of testosterone remained unaffected in the presence of these compounds, demonstrating that they do not interfere with the electrochemical signal under the optimized conditions. This confirms the robustness of the method for application in pharmaceutical formulations.

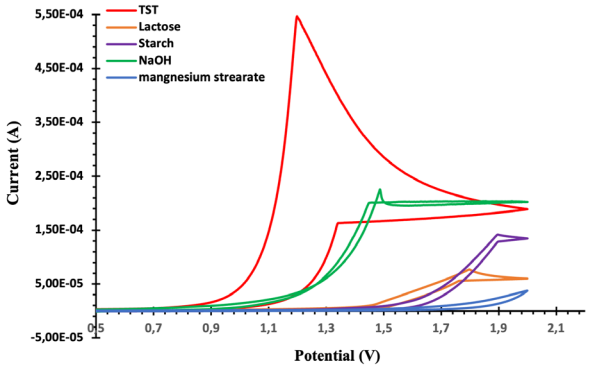

Figure 4. Effect of common excipients (lactose, magnesium stearate, starch) on the electrochemical response of testosterone at the GCE (500 ppm, pH 12.7, 40 °C, scan rate 50 mV·s^{-1}).

Precision and accuracy

The developed cyclic voltammetric method was validated for specificity, linearity, accuracy, and precision [22]. Precision and accuracy were first evaluated by repeated analysis of bulk testosterone at different concentration levels. Eight triplicate measurements were performed within the range of 40–80 mg. The obtained voltammograms showed reproducible shapes (Figure S10 and S11), and the calculated recoveries demonstrated good agreement with the expected values.

To further confirm the method's applicability to real samples, the procedure was applied to a commercial pharmaceutical formulation (Proviron, obtained from local pharmacies). The testosterone content determined by the proposed method was consistent with the labeled amount, as summarized in Table 2.

The recoveries ranged from 99.16% to 101.43%, with relative standard deviations (RSD%) below 2.6%, meeting the criteria for reliable quantitative analysis. These results confirm that the method provides excellent precision and accuracy in both bulk materials and finished dosage forms.

Table 2. Analytical performance of the developed CV method for testosterone determination in bulk and pharmaceutical formulations.

Sample type	Nominal (mg)	Found (mg)	Recovery (%)	RSD (%)
Bulk	20.00	18.70	101.27	2.51
	40.00	38.63	99.16	1.17
	80.00	78.46	99.28	1.02
Pharmaceutical formulation	40.00	39.26	101.43	1.27
	60.00	59.86	99.88	0.95
	80.00	79.69	101.14	0.48

As shown in Table 2, recoveries for bulk testosterone ranged from 99.16% to 101.27% with RSD values below 2.6%. For pharmaceutical formulations, recoveries ranged from 99.88% to 101.43%, with RSD not exceeding 1.3%. These results demonstrate the high accuracy and precision of the proposed method, confirming its suitability for routine quality control of testosterone in both raw materials and dosage forms.

Conclusion

This study demonstrated the successful application of cyclic voltammetry for the determination of testosterone in bulk and pharmaceutical formulations. The method showed excellent linearity over the concentration range of 3.47×10^{-5} – 3.47×10^{-4} M, with a low detection limit (LOD = 2.08×10^{-5} M) and quantification limit (LOQ = 6.30×10^{-5} M). Thermodynamic analysis confirmed the spontaneous nature of the electro-oxidation process, involving a two-electron transfer. The method was further validated by precision and accuracy studies, yielding recoveries close to 100% and RSD values below 2.6%. Importantly, common pharmaceutical excipients did not interfere with the assay, confirming its selectivity. Overall, the results indicate that cyclic voltammetry provides a rapid, sensitive, and cost-effective tool for testosterone quantification, with potential applications in routine quality control and pharmaceutical analysis. Future work should explore its applicability to biological matrices and expand the method's use for simultaneous determination of testosterone with structurally related compounds.

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