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Walaa Saber

walaasaber727@gmail.com

Egyptian Drug Authority

Hassan. A.M. Hendawy

Egyptian Drug Authority

Abdalla Shalaby

Zagazig University

Rania A. Sayed

Zagazig University

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Nano-ZnO Electrode Engineering Combined with Definitive Screening Design for Sustainable Electrochemical Sensing of Tedizolid Phosphate

Walaa Saber^{*1}, Hassan. A.M. Hendawy¹, Abdalla Shalaby², Rania A. Sayed²

¹ Egyptian Drug Authority, Cairo, Egypt

² Department of Analytical Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt

*Corresponding author: Walaa Saber, Egyptian Drug Authority (EDA), P.O. Box 29, Giza, Egypt

Email: walaasaber727@gmail.com . Fax: (+2) 0233379445

Abstract

The burgeoning threat of multidrug-resistant Gram-positive infections demands innovative approaches to antimicrobial therapy. While the development of novel agents often lags behind the emergence of resistance, Tedizolid phosphate (TDZ), a novel oxazolidinone, offers promising therapeutic potential as it demonstrates activity against multidrug-resistant pathogens. To address the need for TDZ quantification, we present a novel robust green voltammetric method employing the combined strengths of nanotechnology and chemometrics. Differential pulse voltammetry (DPV) measurements were performed using Nano ZnO-CPE. A dual response definitive screening design (DR-DSD) was utilized to achieve optimal performance. This approach effectively pinpoints crucial factors, builds a detailed model of their interactions, optimizes conditions for desired outcomes, and ensures reliable performance by minimizing external influences, all to achieve analytical goals. The method undergoes validation in accordance with ICH guidelines and demonstrates exceptional accuracy over a concentration range of 7.49 -81.07 ng mL⁻¹ for TDZ. To assess the ecological impact, two greenness evaluation tools were employed, including the Analytical Greenness Calculator (AGREE) and the Green Analytical Procedure Index (GAPI). The developed method was successfully applied to analyze the dosage form and statistically compared to a previously published HPLC

method. No significant differences between the two methods were observed at a 95% confidence level, demonstrating the accuracy and reliability of our proposed approach. This robust and time-efficient procedure provides a valuable analytical tool for the quantification of TDZ in pharmaceutical dosage forms. Additionally, the method is environmentally friendly, as evidenced by the greenness evaluation results.

Keyword: Design of experiment (DoE); Differential pulse voltammetry; Electrochemical sensor; Nanotechnology.

Introduction

The escalating prevalence of multidrug-resistant Gram-positive bacterial infections presents a significant clinical challenge. The advancement of novel antimicrobial therapies is struggling to keep pace with the rapid evolution of resistance mechanisms. Tedizolid phosphate (TDZ), a next-generation oxazolidinone derivative, exhibits promising therapeutic activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) species, Figure 1 [1,2].

Precise quantification of TDZ in pharmaceutical formulations is essential for maintaining therapeutic efficacy, ensuring patient safety, and adherence to established quality assurance protocols. While various analytical techniques have been utilized for TDZ quantification, including mass spectrometry coupled UPLC-MS/MS [3], HPLC-MS/MS [4], HPTLC [5], spectrofluorometry [6], and potentiometry [7].

Voltammetry offers a direct method for assessing the electrochemical characteristics and gaining insights into the redox behavior of the analyte. To enhance the performance of this technique, zinc oxide nanoparticles (nano-ZnO) were incorporated, leading to improved sensitivity, accelerated reaction kinetics, and enhanced analyte selectivity [8,9].

Optimization of electroanalytical methods often necessitates advanced methodologies due to their complex nature. Design of Experiments (DoE) was utilized to model the relationship between input parameters and output responses using mathematical frameworks [10,11]. Specifically, Definitive Screening Design (DSD) was employed for the efficient identification of influential factors, thereby focusing optimization efforts on critical parameters with a reduced number of experimental runs [12-14]. Furthermore, principles of robust design were integrated to minimize the impact of uncontrollable variables on the final analytical outcome [15,16]. Consequently, a dual response approach (DR) was applied to simultaneously optimize both the mean and standard deviation of the analytical response. The developed method exhibited environmentally

benign characteristics compared to conventional techniques, aligning with the principles of sustainable chemistry. This greener approach minimizes environmental burden and promotes responsible laboratory practices, establishing a new benchmark. Importantly, its successful application in quantifying TDZ in pharmaceutical samples highlights its practical utility as a valuable tool for quality control and pharmaceutical analysis.

This study aims to fill the gap in the literature by developing and validating a novel voltammetric method for the determination of TDZ, integrating DR-DSD and green chemistry principles. To the best of our knowledge, this is the first reported voltammetric method for TDZ quantification. The application of DR-DSD ensures method robustness and reliability, while adherence to green chemistry principles reduces environmental impact. This method offers a precise, sustainable, and efficient tool for TDZ quality control in pharmaceutical formulations, contributing to the advancement of modern analytical methodologies.

Experimental

Instrumentation

All voltammetric experiments were carried out using a Metrohm computrace voltammetric analyzer model 797 VA (Metrohm, Herisau, Switzerland) equipped with three electrodes: a working electrode, Ag/AgCl electrode as reference electrode and a platinum electrode as auxiliary electrode. HPLC analysis was performed using A Dionex UltiMate 3000 HPLC system (Thermo Scientific) equipped with a TCC-3X00(RS) column compartment oven, MWD -30000 (RS) detector, LPG-3400SD Quaternary pump and an UltiMate ACC-3000 autosampler. The chromatographic experiment was performed using gradient mode on a Phenyl-hexyl reversed-phase HPLC column (250 mm × 4.6 mm, 5 μm). The pH measurements were performed with a JENWAY 3510 pH meter (Jenway, Staffordshire, UK). A high-resolution image of the electrode morphology was obtained using a scanning electron microscope (SEM) operating at an accelerated voltage of 5 kV (JSM-7610F, JEOL) (Tokyo, Japan).

Software

- VA Computrace version 1.3.1 (for voltammetric experiments and data acquisition).
- Chromeleon version 7.1 (for chromatographic experiments and data acquisition).
- Design-Expert® trial version 10.0 (for experimental designs and statistical data analysis).

- MOPAC software in conjunction with MP7 and Hukel MM94 software (for molecular orbital calculations).

Reagents and chemicals

- Tedizolid phosphate (TDZ) (99.8 % purity) was supplied by Marcyrl Pharmaceutical Industries, Cairo, Egypt.
- Graphite powder (particle size 20 μm) and Nano ZnO (Particle size <100 nm) were purchased from Sigma–Aldrich (United States).
- β -cyclodextrin, glacial acetic acid, boric acid, sodium hydroxide, orthophosphoric acid (85%), disodium hydrogen phosphate, trimethylamine, sodium dodecyl sulfate, acetonitrile and paraffin oil (99%) were Purchased from Merck (Germany).
- Ultrapure water was obtained from a Milli-Q[®] system.
- Zolidocyryl[®] tablets (Batch number:173292, Marcyrl Pharmaceutical Industries, Cairo, Egypt.), labeled to have 200 mg TDZ per tablet, were purchased from local pharmacies.

Preparation of electrolyte solution

Britton-Robinson (BR) buffer solution was chosen as a supporting electrolyte for the pH study. This solution was prepared by mixing 0.04 M of acetic acid, boric acid, and orthophosphoric acid with pH adjusted from 2 to 11 with 2M NaOH solution [17].

Standard solutions and sample preparation

Standard solution of TDZ (1.0×10^{-3} M) was prepared by accurately dissolving 45.03 mg of TDZ in a 100 mL volumetric flask containing 5 mL of 0.1M NaOH, sonicated for 5 minutes then the volume was completed to the mark with the ultrapure water. The working solution of TDZ (1.0×10^{-5} M) was prepared by further dilution with water. It was freshly prepared every day of measurement.

For tablet analysis, ten zolidocyryl[®] tablets were pulverized, and a weighed amount of the powder containing 11.26 mg of TDZ was transferred to a 25 mL volumetric flask. The excipients were separated by filtration; further appropriate dilutions were done. It was freshly prepared on every day of measurement.

Electrodes preparation

Experiments were achieved using the following electrodes:

Carbon paste electrode (CPE)

Carbon paste electrode (CPE) preparation involves mixing 0.5 g of graphite powder with a particle dimension of 20 μ m with 0.3 mL paraffin oil using a mortar and pestle. The resulting composite carbon paste was then packed into the hole of an insulin syringe body that had a diameter of 3 mm. Inside the syringe, there was a copper wire that contacted the apparatus. The tip of the electrode was polished until it had a shiny appearance using an emery paper 3000.

Modified Nano-ZnO electrode (Nano ZnO-CPE)

Nano ZnO-CPE was prepared in the same way by weighing 0.01 g Nano ZnO and combining it with 0.49 g graphite powder to get a final mixture weight of 0.5 g, followed by mixing in a mortar with 0.3 mL paraffin oil. Finally, a piece of the paste was placed into the 3 mm diameter hole in the insulin syringe body, which included a copper wire that contacted the apparatus.

Influence of the pH of supporting electrolyte

To elucidate the influence of pH on peak current, cyclic voltammetry (CV) was employed across a pH range of 2.0-11.0. The resulting voltammograms were meticulously analyzed to evaluate the variations in peak current as a function of pH.

Effect of scan rate on peak current and potential

The influence of the scan rate ($\mathcal{V}$) on both the peak current intensity (I_p) and peak potential position was investigated from 0.02 to 0.16 V s⁻¹ using cyclic voltammetry.

Experimental conditions optimization using Definitive Screening Design (DSD)

The critical method parameters (CMPs) and the critical method attributes (CMAs) were studied. Definitive Screening Design (DSD) served as the initial tool for unraveling the effects of CMPs on CMAs. Five CMPs (modifier, voltage step, pulse amplitude, voltage step time and surfactant) were studied, Table 1. Two CMAs were targeted in the optimization process. CMAs were the mean of the TDZ oxidation peak current ($M I_p$) and standard deviation of the TDZ oxidation peak current ($S I_p$). The goal for ($M I_p$) was to maximize the average peak current, resulting in stronger signals and potentially improved sensitivity. While ($S I_p$) was targeted to minimize the variability of peak current measurements, leading to greater precision and reproducibility in the analysis. Optimizing for both CMAs simultaneously allowed for maximizing signal strength while maintaining control over measurement consistency. This dual approach ensured reliable and accurate quantification of the target analyte. The experimental matrix was composed of 14 runs (Table 2).

After the selection of CMPs, DR-DSD was conducted. We collected repeated samples for each run ($n = 3$), where the mean and the standard deviation were entered as responses[18].

Electrochemical procedure

The Ag/AgCl reference and Pt electrodes were rinsed with ultrapure water before each electrochemical measurement. The carbon paste electrode (CPE) surface was manually smoothed against weighing paper to ensure a reproducible surface. Voltammetric measurements were conducted using the CPE as the working electrode after obtaining a stable voltammogram in Britton-Robinson (BR) buffer solution. For solution preparation, precise volumes of the standard TDZ solution were transferred into 15 mL measuring cylinder and diluted to the mark with 0.04 M BR buffer at the optimal pH of 2. A pre-concentration step of 5 seconds was employed at an accumulation potential of 0.0 V under continuous agitation at 2000 rpm. Following a 10-second quiescent period, voltammetric scans were initiated. Cyclic voltammetry (CV) was performed within a potential range of 0.2 to 1.2 V at a scan rate of 0.1 V s⁻¹ and pH 2. Differential pulse voltammetry (DPV) parameters were set as follows: pulse amplitude, 0.05 V; scan rate, 0.1 V s⁻¹; voltage step time, 0.079 s; voltage step, 0.005 V; and pulse time, 0.04 s. DPV scans were conducted over an oxidation potential window spanning from +0.4 to +1.2 V. All electrochemical experiments were maintained at a controlled temperature of 25 ± 1 °C. Then, the regression equation was derived and used to calculate the mean concentration of each sample.

Method Validation

This method employed the peak oxidation current (I_p) as the analytical response and underwent validation in accordance with ICH guidelines[19]. Key performance parameters including selectivity, linearity range, limit of detection (LOD), limit of quantification (LOQ) precision, and accuracy were thoroughly evaluated.

Results and Discussion

Electrochemical investigation utilizing differential pulse voltammetry (DPV) revealed a prominent anodic peak at a potential of 0.82 V in the presence of the analyte. The well-defined anodic peak only appeared when the analyte was present in solution and thus represented the electrooxidation of TDZ under the given experimental conditions, Figure 2. The absence of the anodic peak in the blank confirmed that it was attributed to TDZ oxidation. The well-defined potential and current response are strong evidence of the effectiveness of DPV in the detection of TDZ oxidation, outlining the sensitivity and reliability of the

method for electrochemical characterization in complex systems.

Characterization of the Nano ZnO-CPE

Electrochemical characterization

The cyclic voltammetry results provide detailed insights into the electrochemical behavior of the Nano ZnO-CPE electrode as a working electrode in a three-electrode electrochemical cell. Figure 2 illustrates cyclic voltammograms recorded at various scan rates ranging from 10 to 100 mV/s. At a low scan rate of 10 mV/s, ΔE_p was determined to be 0.071 V, which decreases to 0.065 V at an increased scan rate of 180 mV/s. On the other hand, i_{pc}/i_{pa} changes from 1.02 to 1.09 within this range of the scan rate. These values have indicated that $K_3Fe(CN)_6$ exhibits electrochemically reversible redox with the ZnO-CPE electrode. The dependence of peak separation on the scan rate shows that the kinetics of electron transfer between Fe(III)/Fe(II) redox sites and the ZnO-CPE surface control the reversibility of the system. However, the deviation in ΔE_p from the ideal value of 59 mV and i_{pc}/i_{pa} ratio from 1.00 indicates the realistic impact of electrode material and system-specific parameters. A plot of the peak currents as a function of the square root of the scan rate in Figure 3 gives a straight line, demonstrating that the electrochemical behavior is under diffusion control as described by the Randles-Sevcik equation. Diffusion of $K_3Fe(CN)_6$ at carbon-based electrodes has been reported to lead to semi-infinite planar diffusion due to the overlapping of its diffusion layers, and a similar behavior was seen for ZnO-CPE herein, mainly at its porous electrode surface. Such diffusional behavior of the electroactive species resembles that of carbon nanotube bundle electrodes, where edge-to-edge overlapping of diffusion layers directs the species onto inner pore surfaces. However, with more rapid scan rates, 100 mV/second part of the electrode area is accessible to the Fe(III)/Fe(II) redox couple due to the short time scale of the experiment. Therefore, diffusion hardly reaches the innermost pores, which makes the large electroactive surface area confined in the pores less accessible.

Figure 3b further illustrates the observations made: linear equations of oxidation ($y = 3 \times 10^{-6}x + 2 \times 10^{-6}$, $R^2 = 0.9882$) and linear equations of reduction ($y = -4 \times 10^{-6}x - 2 \times 10^{-6}$, $R^2 = 0.9867$). These results agree with the particular porous structure of the ZnO-CPE supporting excellent ability for electron transfer.

Finally, based on the Randles-Sevcik equation [20-22] equation

$$I_p = (2.69 \times 10^5) A n^{3/2} D^{1/2} C v^{1/2} \quad \text{Equation (1)}$$

Where I_p is the anodic peak current; n is the number of electrons transferred; A is the electrode surface area

(cm²); D is the diffusion coefficient (cm² s⁻¹); v is the scan rate (V s⁻¹); C is the K₃Fe(CN)₆ concentration (mol cm⁻³). The real electroactive surface area of ZnO-CPE was determined and calculated to be 0.348 cm² in a solution containing 1.0 mM [Fe(CN)₆]^{3-/4-}. In light of such enhanced surface area, it would thus be easier for the ZnO-CPE to offer active sites with fast electron transfer kinetics. Consequently, better conduction paths become available due to enhanced electron mobility, thereby characterizing the ZnO-CPE as one of the most efficient and effective advanced material electrodes compared with traditional ones for advanced applications. The inset shows the cyclic voltammograms obtained with a 20 mM potassium ferricyanide probe solution at varying scan rates. The main panel presents the relationship between the anodic peak current and the scan rate. The linearity of this relationship confirms a surface-controlled process, and the slope is proportional to the active surface area of the modified electrode.

SEM characterization of Nano ZnO-CPE electrode

Figure 4 shows the electrode architecture that is composed of spherical layers of uniformly dispersed nanozinc oxide (nano ZnO) particles with bulk and surface of electrode. The compactness of the ZnO particles in the electrode increases the inter-sheet contact, hence enhancing mechanical stability and electrical conductivity. This is further facilitated by the inherent porosity of the structure, which increases not only the surface area available for analyte immobilization but also allows electrolyte penetration through microvoids formed within the graphite matrix due to densely packed nano ZnO particles. The reservoir thus provided by this configuration becomes important for the storage of the electrolyte, hence stabilizing the analyte during the electrochemical reaction. Furthermore, the aligned layered morphology of nano ZnO particles significantly enhances the active surface area available for redox reactions and accelerates electron transfer rates. Moreover, the band gap of nano ZnO is one of the most important factors in determining the electronic properties and reactivity of nano ZnO. Conventionally, zinc oxide has a wide band gap of about 3.37 eV, which can be engineered by adopting different synthesis methods to optimize performance in applications related to sensors. Modification of the band gap alters charge carrier dynamics, hence increasing the efficiency of electron transfer processes involved in electrochemical reactions. Therefore, band gap tailoring by Nano structuring improves conductivity and allows better interaction with the analytes, enhancing general device performance when using nano ZnO as an active sensor.

Influence of the pH of supporting electrolyte

The pH of the electrolyte medium is one of the variables that commonly and strongly influence the shape of the voltammogram. Hence, it was important to investigate the effect of the pH on the electrochemical behavior of the drug [23]. The influence of pH on the peak current was examined using CV over the pH range of 2-11 using 0.04 M BR buffer. A linear correlation between the peak potential and solution pH was obtained revealing that a proton has taken part in the reaction. The following linear equation was obtained

$$E(\text{mV}) = -0.0503 \text{ pH} + 1.0363 \quad \text{Equation (2)}$$

It was found that a negative shifting of the oxidation peak occurred by increasing pH and the maximum peak current was obtained at pH 2 at CPE, Figure 5. Hence pH 2 was used as an optimum pH value throughout the whole study.

3.3 Effect of scan rate on peak current and potential

The scan rate studies were conducted to evaluate if the process at the selected electrodes was under diffusion or adsorption-controlled process. The effect of the scan rate ($\mathcal{V}$) on the peak current (I_p) and potential was evaluated using cyclic voltammetry, over the scan range 0.02–0.16 Vs^{-1} . The study revealed that the peak current increased with an increase in the peak potential towards the positive direction upon increasing the scan rate. Quantitative assessment of the relationship between the logarithm of the peak current ($\log I_p$) and the logarithm of the scan rate ($\log \mathcal{V}$), as illustrated in Figure 6, revealed a strong linear correlation. The experimentally determined slope of this linear dependence, approximating 0.5, aligned closely with the theoretically predicted value for an electrochemical reaction under diffusion-limited conditions. The linear regression analysis yielded the following equation:

$$\log I_p (\text{A}) = 0.5923 \log \mathcal{V} (\text{Vs}^{-1}) + 6.6602, \quad (R^2 = 0.989) \quad \text{Equation (3)}$$

Mechanism of the proposed oxidation of TDZ using Nano ZnO-CPE

The prevailing electrooxidation mechanisms governing the behavior of TDZ primarily revolve around the oxidation of the nitrogen atom within the aromatic 5-ring structure, possessing a p-lone pair moiety. This choice hinges on the unique properties of a p-lone pair of electrons on the terminal nitrogen atom, which forms a bond with a methyl group and experiences minimal steric hindrance. In-depth molecular orbital calculations were conducted on TDZ molecules utilizing MOPAC software in conjunction with MP7 and Hukel MM94 software. These calculations elucidated the region of highest electron density, situated at the nitrogen atom of the terminal amine, where no withdrawing group was present. Furthermore, resonance

phenomena were observed, and the tutors induced a rearrangement of bi-bonds within the adjacent five-membered ring. The oxidation process, integral to the electrooxidation mechanism, unfolds through a multistep mechanism involving the transfer of one proton and two electrons. This redox mechanism, albeit multistep, can be effectively summarized as a one-step mechanism, as succinctly illustrated in Figure 6.

DR-DSD strategy for experimental conditions optimization

Optimizing experimental conditions often requires testing a large number of variables, which can be time-consuming and resource intensive. DSD are a powerful tool for efficiently exploring the effects of multiple factors in an experiment while minimizing resource consumption. Dual response offers a powerful approach to simultaneously optimize both the mean and variance of a response variable [16,18]. This allows for achieving desired outcomes across multiple dimensions, leading to more robust and reliable results. To implement DR-DSD effectively, these key steps outlined were followed:

- Define the responses, design the experiment, develop the DSD, simultaneous optimization and validation and confirmation
- Fitting response surfaces for the mean (μ) and standard deviation (σ) responses separately.

Definitive Screening Design (DSD) is characterized by its concise nature, requiring a significantly smaller number of runs compared to a full factorial design. This results in substantial resource savings in terms of time, materials, and labor compared to a complete factorial design. Despite their reduced size, DSD has remarkable ability to estimate all main effects and some interactions between factors with high accuracy.

This leads to identification of the most significant factors impacting the outcome of the experiment, allowing to focus the resources on further optimization efforts without wasting time on irrelevant factors [24,25].

DSD finds out the significant factors in the studied responses. The F-value of 13.91 and 234.97 for the mean of the oxidation of peak current and standard deviation of the oxidation peak current respectively indicated that the model was significant, Tables 3 and 4. The modeling of three CMPs using a DSD was generated.

The data were fitted to a first-order or a quadratic equation to produce predictive models relating the CMPs and CMAs as shown in Table 5. To achieve a parsimonious model that accurately reflects the underlying mechanism, unimportant terms were systematically removed using forward model selection guided by the corrected Akaike Information Criterion (AICc)[15]. The model terms of CMAs were statistically significant (p -value < 0.05) indicating good models. ANOVA results for the reduced models are presented in Tables 3

and 4. Statistical parameters such as adjusted R^2 , predicted R^2 and adequate precision are presented in Table 5. Predicted R^2 measures the variation in new data explained by the model, and their values were in agreement with the adjusted R^2 values. Adequate precision measures the experimental signal-to-noise ratio [8, 11]. Figure.7 shows the 3D contour plots of CMAs of DR-DSD. In the presence of multiple response processes, Derringer's desirability function (D) was used; it was based on a scale of (0-1). The optimization of D led to the following optimum voltammetric conditions: 10 mg modifier, voltage step =0.005, pulse amplitude =0.050, and voltage step time= 0.079 s in the absence of surfactant. Finally, five voltammetric measurements were analyzed to confirm the optimum conditions; the differences between the experimental and the predicted values were < 4.2%.

Method Validation

The method was thoroughly validated in accordance with (ICH) guidelines [19]. The oxidation peak current (I_p) was the analytical response.

Linearity and sensitivity

The voltammetric behavior of TDZ at the Nano ZnO-CPE was examined under the optimal selected conditions. Different concentrations of TDZ solution was prepared and added separately to the pH 2.0 buffer solution in the voltammetric cell using a micropipette. The modified electrode was then immersed in the solution. A calibration curve was constructed by correlating the TDZ concentration ($\mu\text{g mL}^{-1}$) with the measured anodic peak current (I_p , μA), and the regression equation was determined, Figure 8. The results of linearity were presented in Table 6. The high value of the correlation coefficient indicated the excellent linearity of the proposed method. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated according the equations:

$$\text{LOD} = 3.3 \text{ SD/S} \quad \text{Equation (4)}$$

$$\text{LOQ} = 10 \text{ SD/S} \quad \text{Equation (5)}$$

where S represents the slope of the linear calibration curve and SD represents the standard deviation of the blank. LOD and LOQ were estimated to be 1.2 and 3.7 ng mL^{-1} , respectively indicating the high sensitivity of the proposed method, Table 6.

Precision and accuracy

To validate the method's precision, repeatability and intermediate precision were assessed. Samples were

injected three times a day to examine intra-day precision (repeatability). The same was done on three separate days to evaluate the precision between each day (intermediate precision). The method's accuracy was also examined through determination of three TDZ concentrations three times. The results confirmed the proposed strategy's excellent precision and accuracy, Table 6 and 7.

Reproducibility and stability of Nano ZnO-CPE

Differential pulse voltammetry (DPV) measurements were performed with three different Nano ZnO-CPE to determine their reproducibility. The relative standard deviation value was 2.84 % for TDZ. Stability is a key factor in the electrochemical sensor development; it was examined by keeping the electrode in the refrigerator at 4 °C for a definite period. DPV measurements were performed periodically for 3 weeks. The voltammetric response decreased to only 4.7. The results indicated that Nano ZnO-CPE had long-term stability.

Application to pharmaceutical tablets and statistical analysis

The developed analytical method was successfully applied to determine the content of tedizolid phosphate (TDZ) in Zolidocryl tablets, Table 8. The assay results obtained were compared with the reported HPLC method [26] indicating no significant difference in the results as shown in Table 8.

Greenness Assessment

The pursuit of green analytical methods aims to minimize the environmental impact of analytical procedures while maintaining satisfactory performance. To achieve this goal, a plethora of assessment tools have been meticulously designed and implemented to evaluate the environmental friendliness of analytical methods. These tools meticulously scrutinize various aspects, including reagent quantities, hazard potentials, waste generation, energy consumption, procedural complexity, and the potential for miniaturization and automation [27]. Among these assessment tools, the Analytical GREENness calculator employs a comprehensive approach, rooted in the twelve principles of green analytical chemistry, delivering easily understandable and insightful results. Enriching the evaluative landscape, the Complex Green Analytical Procedure Index (Complex GAPI) meticulously assesses and quantifies the environmental consequences associated with each analytical step [28-30]. For this study, the selected metrics were Analytical Greenness Calculator (AGREE), and Complex GAPI. Table 9 summarizes the green assessment of the proposed and the reported HPLC method [26]. The AGREE score of the proposed method was 0.83 indicating an excellent green method.

Complex GAPI informative three-color icons offer an instant snapshot of the proposed voltammetric method's environmental footprint. The results conclude that the proposed voltammetric method offers an environmental advantage over the reported one and can be employed for routine analysis of the cited drugs without compromising environmental integrity.

Conclusion

A green and efficient voltammetric method was established for TDZ determination, offering eco-friendly, cost-effective, accurate, and robust analysis. The DR-DSD strategy was implemented using DoE to optimize key parameters for enhanced performance in a minimal number of runs. Statistical modeling of the chosen responses revealed a high degree of predictability and tolerance, validating the method robustness. Furthermore, rigorous validation following ICH guidelines confirmed the accuracy and specificity of the proposed procedure. Notably, TDZ quantification in its dosage form demonstrated excellent accuracy with no interference from excipients, solidifying the method suitability for real-world applications.

Additional Information

Supplementary Information to this article can be found online.

Author Contributions

All authors contributed equally to this work.

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Competing interests

The authors declare no competing interests.

Consent to Participate

Not applicable as the study did not include human subjects

Data Availability

All data generated or analyzed during this study are included in this published article.

Table 1: Factors and ranges of CMPs in the DSD

Factor	Type	Low level	High level
Modifier	Continuous	10	50
Voltage step	Continuous	0.0025	0.0075
Pulse amplitude	Continuous	0.025	0.075
Voltage step time	Continuous	0.05	0.15
Surfactant	Categorical	-	+

Table 2. The experimental matrix generated by the Definitive Screening Design for the investigated factors

run	Modifier (mg)	Voltage step (v)	Pulse amplitude (v)	Voltage step time (s)	Surfactant	Response 1 (mean of peak current)	Response 2 (SD of peak current)
1	50	0.0025	0.05	0.05	-	82	0.1
2	10	0.0025	0.075	0.1	-	94	4.6
3	50	0.005	0.025	0.15	-	98	9.39
4	30	0.005	0.05	0.1	-	81	3.76
5	30	0.005	0.05	0.1	+	74	2.27
6	10	0.0075	0.025	0.05	-	89	0.2
7	50	0.0075	0.025	0.1	+	96	3.62
8	30	0.0025	0.025	0.05	+	70	0.4
9	10	0.0025	0.025	0.15	+	93	10.5
10	50	0.0075	0.075	0.05	-	89	0.1
11	30	0.0075	0.075	0.15	-	93	9.39
12	50	0.0025	0.075	0.15	+	98	9
13	10	0.005	0.075	0.0.05	+	90	0.1
14	10	0.0075	0.05	0.15	+	95	10.3

Table 3. ANOVA for reduced model of CMPs for the first response, mean of the oxidation of peak current (M Ip).

Source	Sum of Squares	df	Mean Square	F -Value	p-value Prob > F	
Model	863.26	4	215.81	13.91	< 0.0007	significant
A-modifier	0.40	1	0.40	0.0258	0.8760	
B-voltage step	62.50	1	62.50	4.03	0.0757	
C- voltage step time	324.90	1	324.90	20.95	0.0013	
A ²	475.46	1	475.46	30.65	0.0004	
Residual	139.6	9	15.51			
Cor Total	1002.86	13				

Table 4. ANOVA for selected model of CMPs for the second response, standard deviation of the oxidation peak current (S Ip)

Source	Sum of Squares	df	Mean Square	F -Value	p-value Prob > F	
Model	234.04	3	78.01	234.04	< 0.0001	significant
A-modifier	1.22	1	1.22	3.67	0.0845	
C- voltage step time	227.34	1	227.34	684.72	< 0.0001	
A ²	475.46	1	475.46	30.65	0.0023	
Residual	3.32	10	0.3320			
Cor Total	237.36	13				

Table 5. Models relating the selected responses and their statistical parameters using the corrected Akaike information Criteria (AICc) forward model selection.

CMAs	Regression equation in terms of coded factors*	ANOVA P- Value	Adjusted R ²	Predicted R ²	Adequate precision
M_Ip	$M_{Ip} = 91.825 - 1.925 A + 1000 B + 114 C + 0.03225 A^2$	0.0007	0.986	0.9818	33.23
S_Ip	$S_{Ip} = 0.092 - 0.01745 A - 15.48C + 554.2 C^2$	<0.0001	0.9608	0.9728	2.3638

* Significant factors; M Ip = mean of the oxidation peak current; S Ip = standard deviation of the oxidation peak current; , A=Modifier ; B=Voltage step ; C= Voltage step time ; A² = Modifier² ; C² = Voltage step time²

Table 6. Characteristics of the calibration curves of TDZ using the proposed DPV method

Parameter	Result
Linearity range (ng mL ⁻¹)	7.49 -81.07
Intercept	0.1012
Slope	6.7209
R ²	0.9995
SD of slope	0.0049
SD of intercept	0.0025
Standard error	0.004
Accuracy	99.91
LOD (ng mL ⁻¹)	1.2
LOQ(ng mL ⁻¹)	3.7

Table.7. Results of analytical precision of the optimized voltammetric method for TDZ determination.

Parameter	Taken	Found	Recovery (%)	RSD
Intraday	25	25.08	100.33	1.20
	45	44.97	99.94	0.53
	65	65.20	100.36	0.47
Inter-day	25	25.01	100.05	0.19
	45	44.90	99.80	0.69
	65	64.92	99.88	0.35

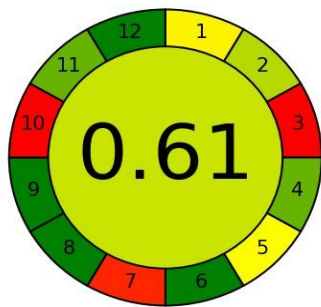
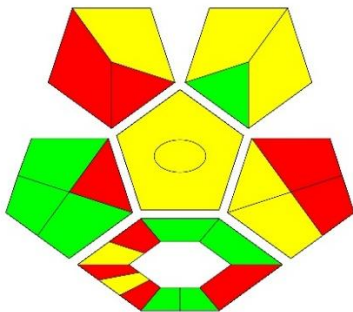
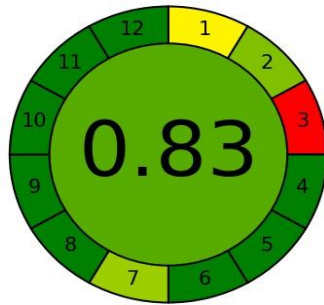
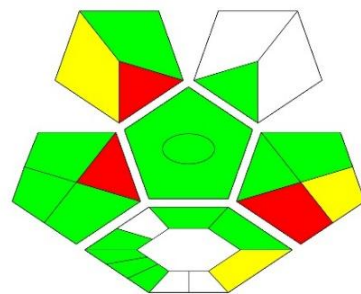
Table 8. Statistical analysis of the results obtained by DPV method for TDZ compared with HPLC method

Parameter	DPV	HPLC ^b
Mean	98.66	99.72
Variance	1.68	1.63
n	6	
Student's t-test (2.23) ^a	1.43	
F-test (5.05) ^a	1.03	

^a values in parentheses represent the tabulated values of F and t at P=0.05

^b HPLC analysis was conducted on a Phenomenex Luna Phenyl-Hexyl (250 mm × 4.6 mm, 5 µm) column at 20°C using gradient elution and flow rate 1 mL/min. A detection was performed at 300 nm. The mobile phase involved β-cyclodextrin buffered solution at pH 7.0 followed by a mixture of equal volumes of distilled water and acetonitrile.

Table 9. Comparison between the proposed and the reported HPLC method [26] greenness for determination of TDZ

Applied method	AGREE	GAPI
Reported HPLC method [26]		
Proposed voltammetric method		

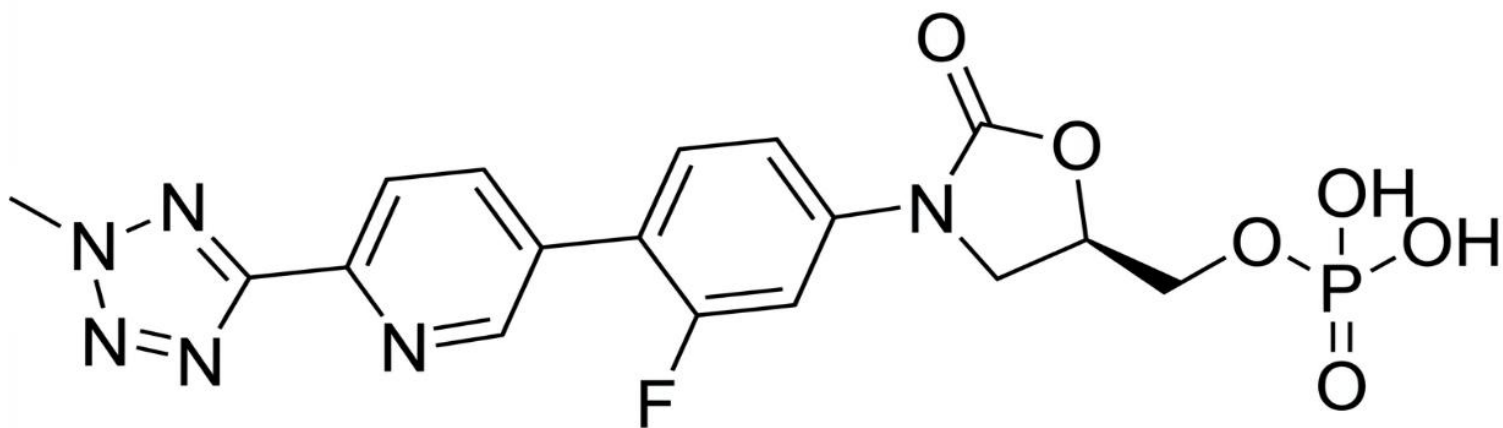


Figure 1. The structure of Tediazolide phosphate

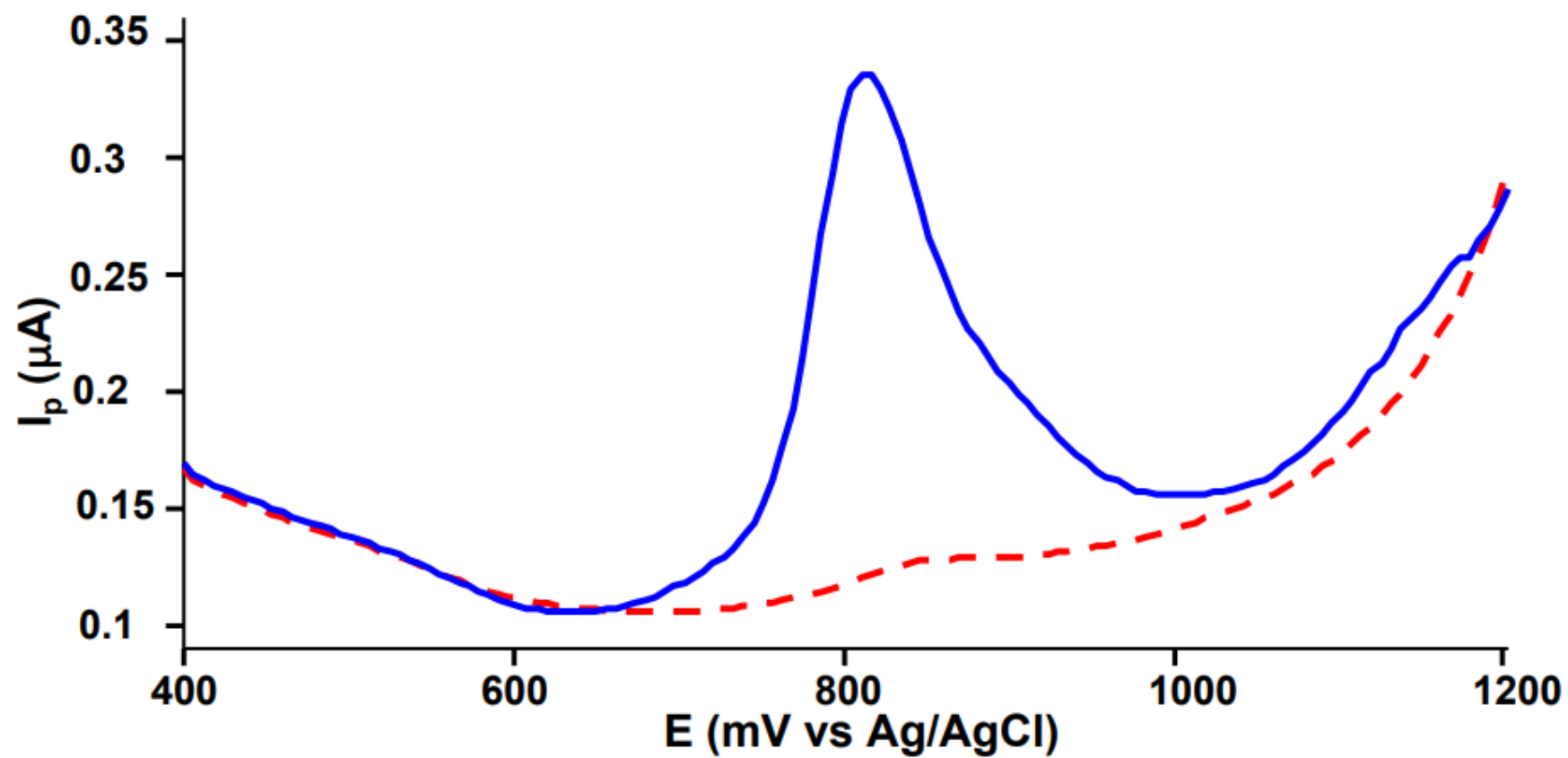


Figure 2. DPV voltammograms of blank (...) and spiked analytes (-).

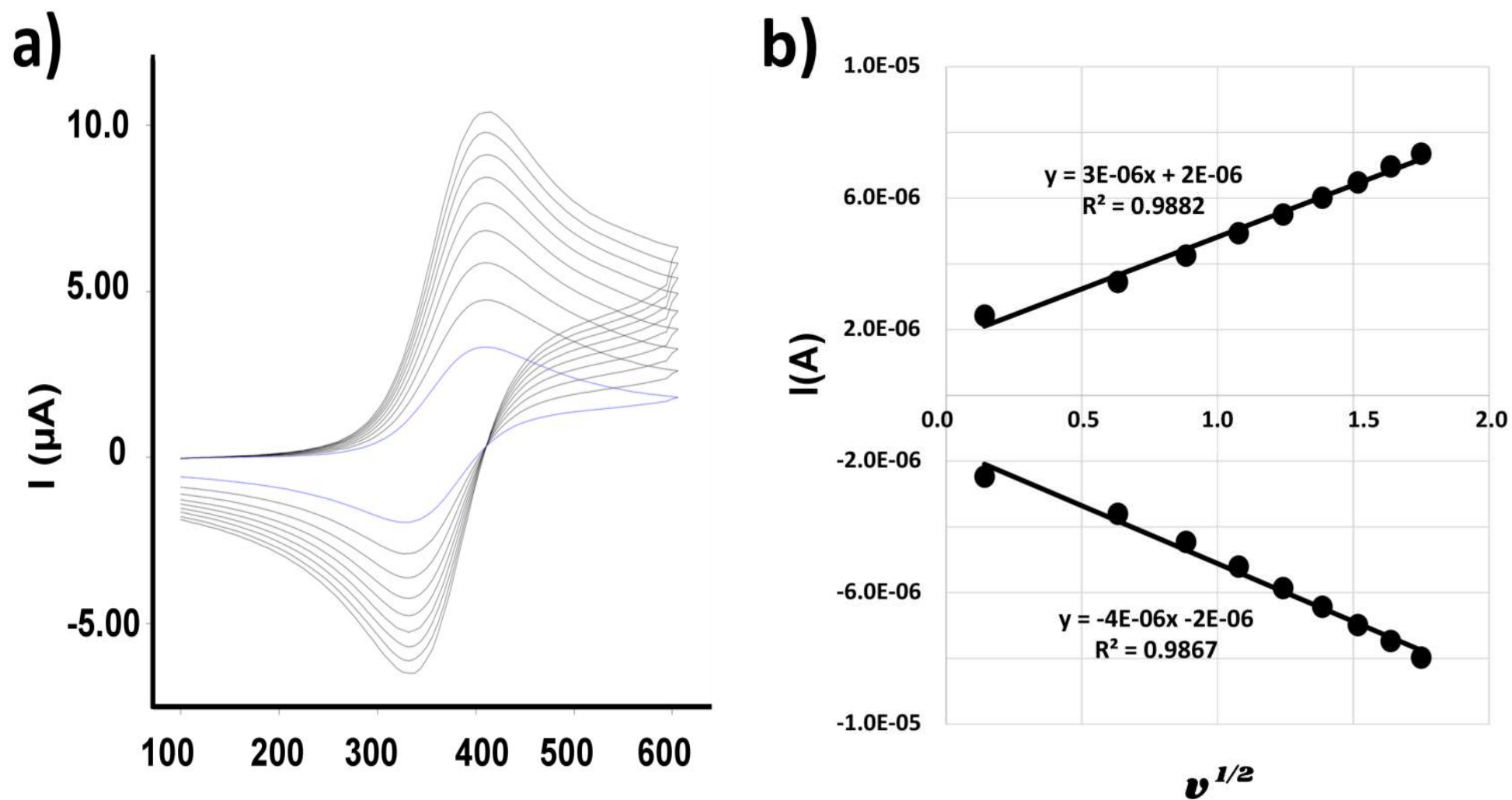


Figure 3. Electrochemical characterization of the Nano ZnO-CPE electrode; a) Cyclic voltammograms at different scan rate values. b) plot of the oxidation peak current vs. square root of scan rate.

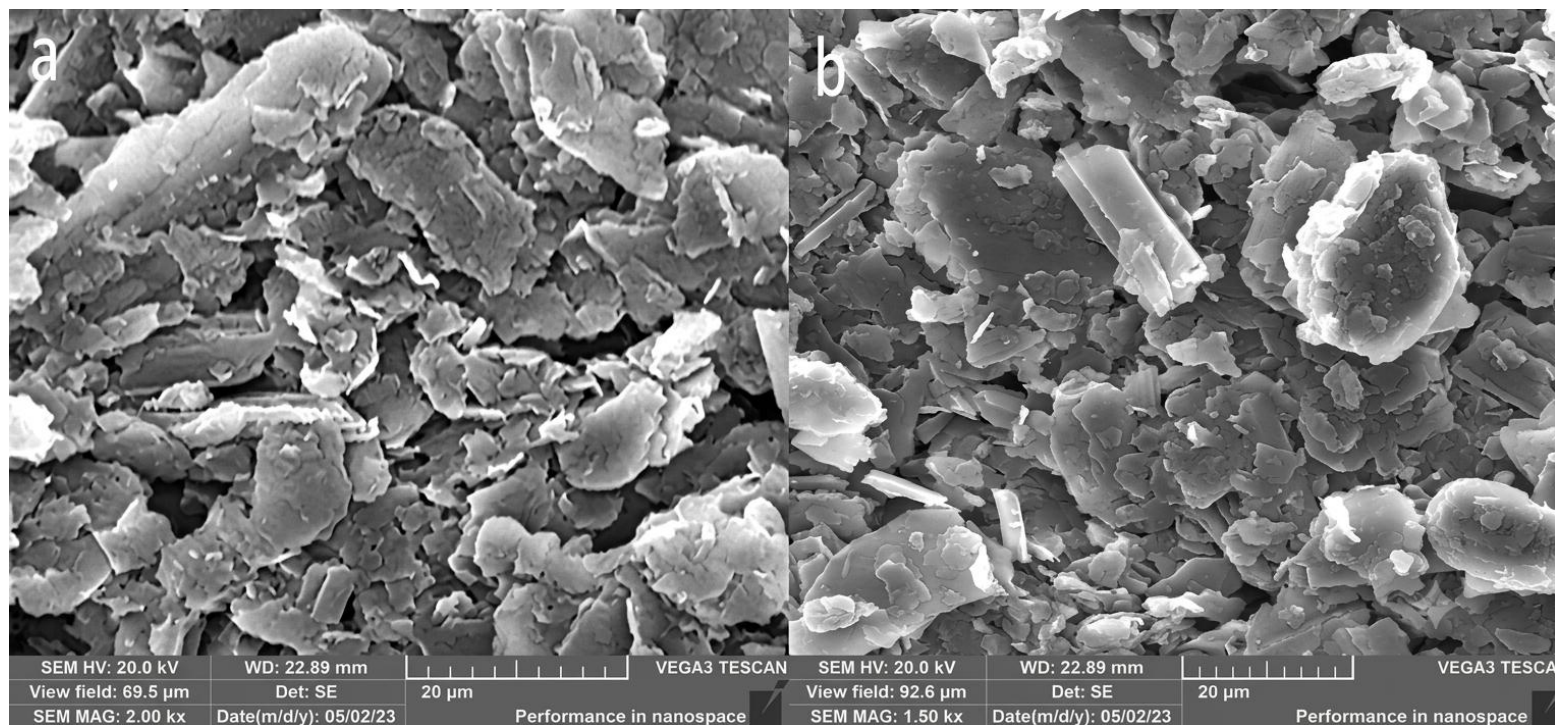


Figure 4. Scanning Electron micrographs of the surface of (a) CPE and (b) ZnO-CPE (Magnification $\times$ 2000).

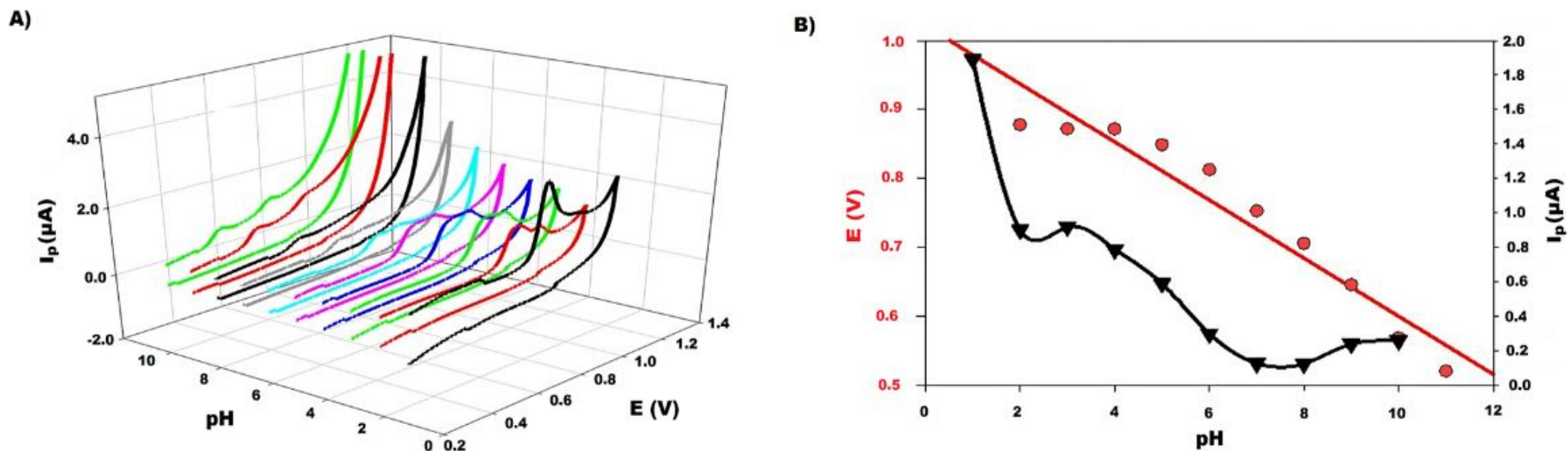


Figure.5 A) CV voltammograms response of $1.49 \mu\text{g mL}^{-1}$ TDZ solution in 0.04 M BR buffer with different pH ranges of 2-11 at Nano ZnO-CPE at scan rate 50mV/s.

B) The relationship between pH with redox potential E(V) and pH with current peak height I(A).

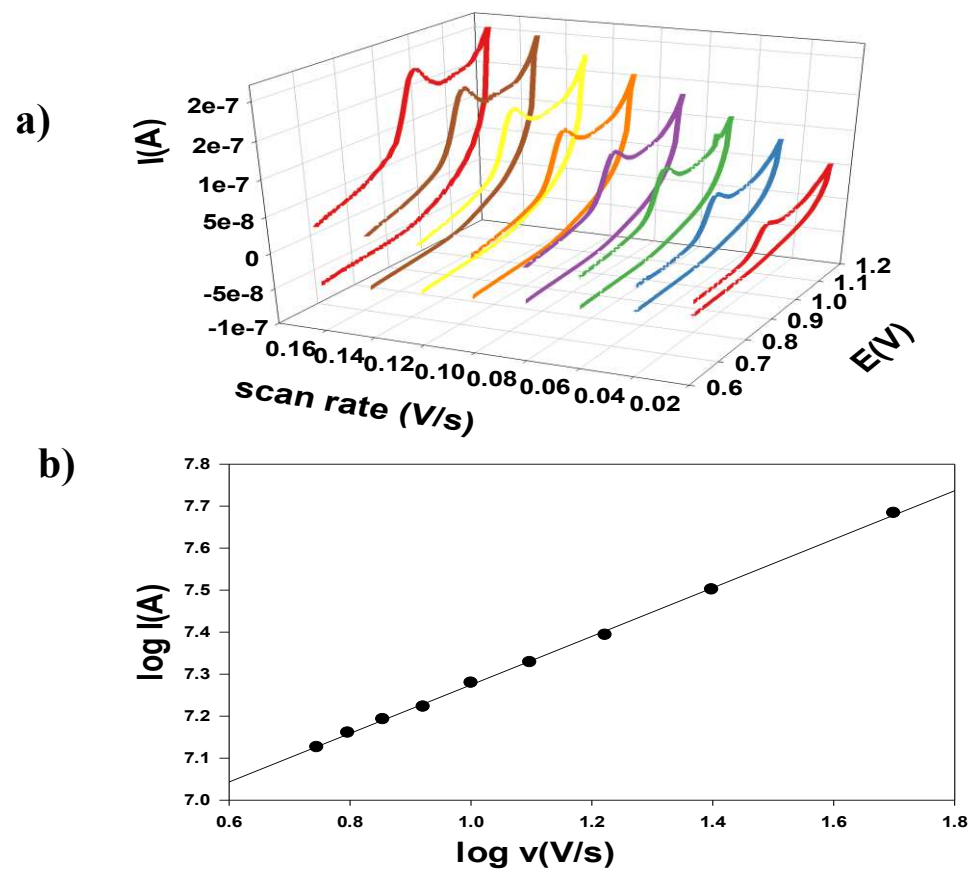


Figure. 6. **a)** 3D cyclic voltammograms recorded at different scan rates ($0.02 - 0.16 \text{ V s}^{-1}$) illustrating the effect of scan rate (v) on the oxidation peak current (I_p) and peak potential (E_p). **b)** Linear plot of $\log I_p$ versus $\log v$, displaying a slope of 0.5923 ($R^2 = 0.989$), confirming a diffusion-controlled mass transport process at the electrode surface.

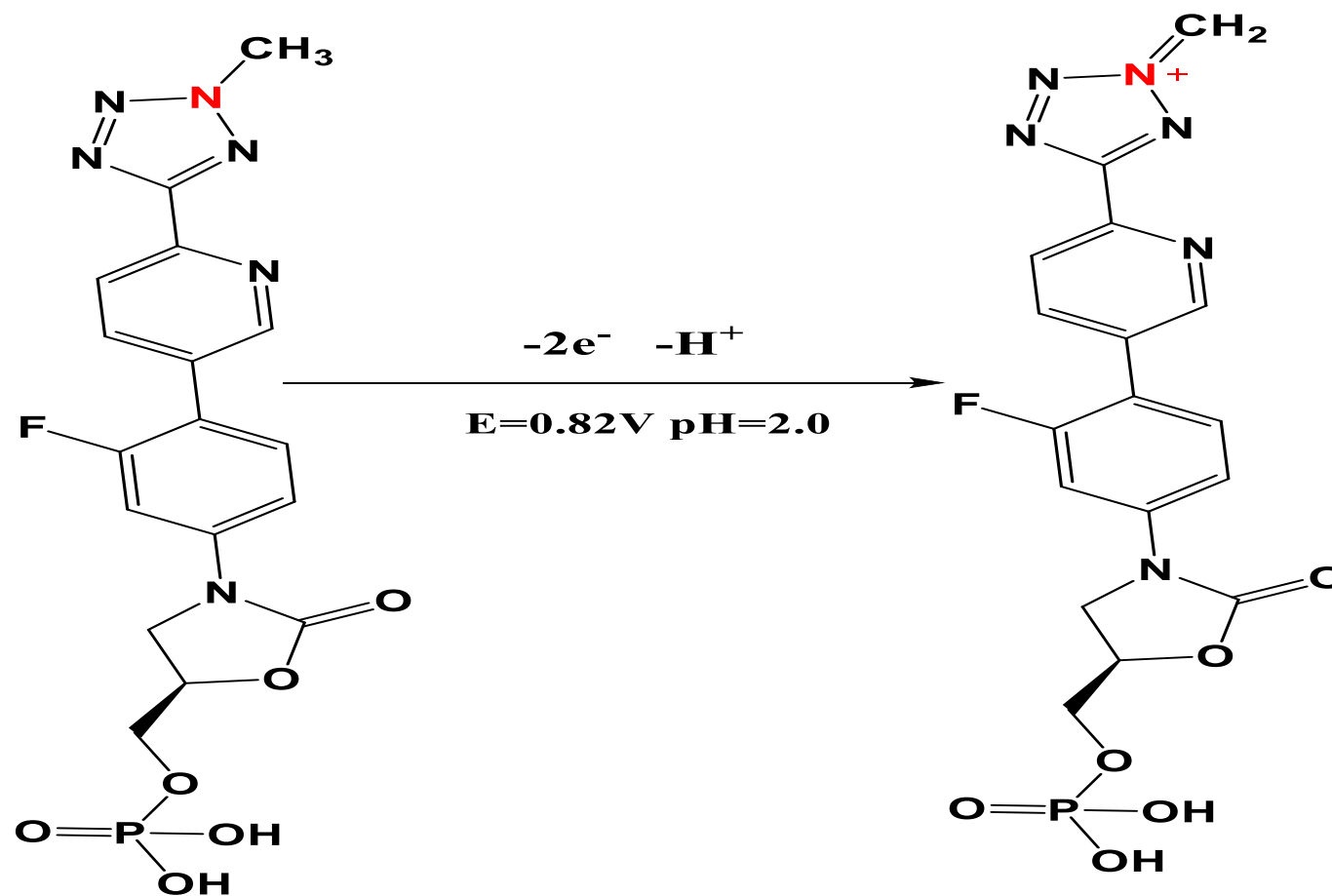
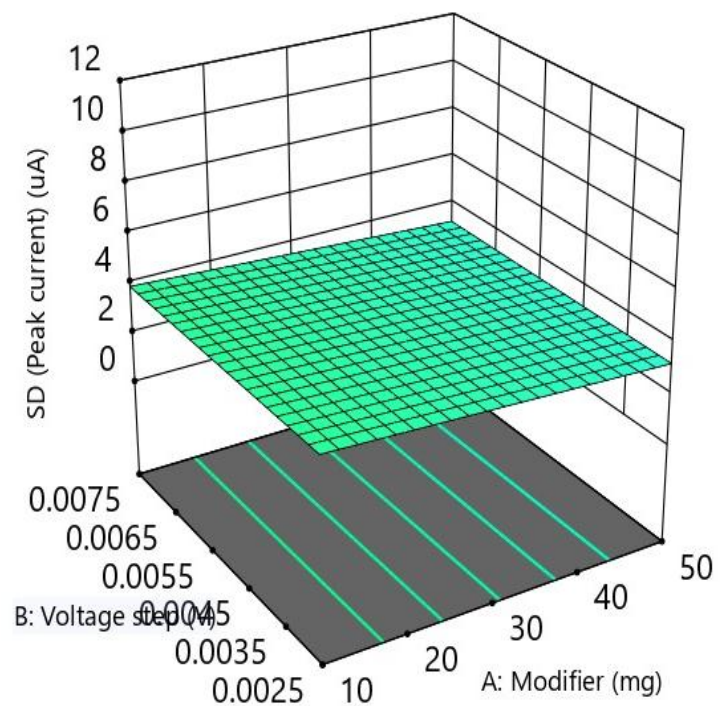
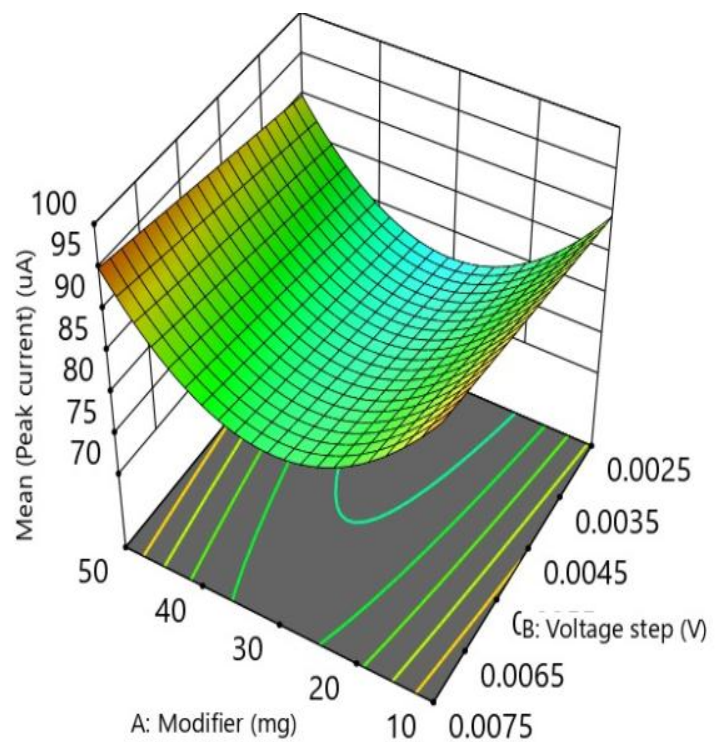


Figure 6. The proposed redox mechanism of TDZ using ZnO-CPE

Figure 5



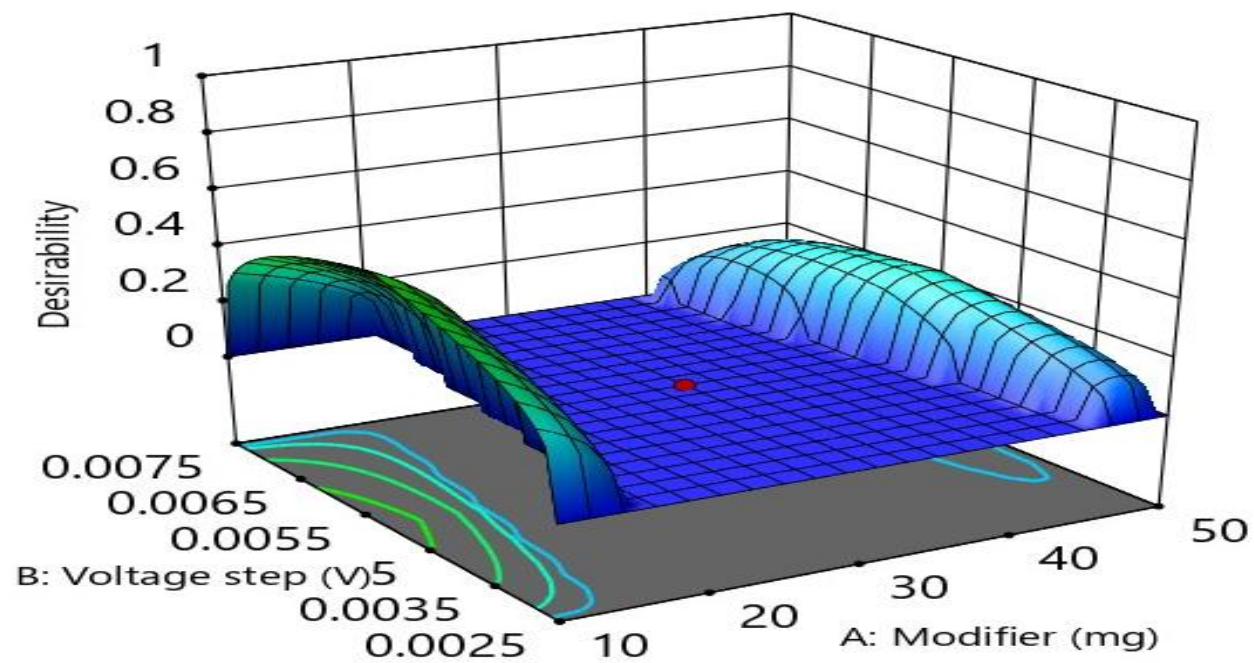


Figure 7. Contour plots of CMAs of DRSM.

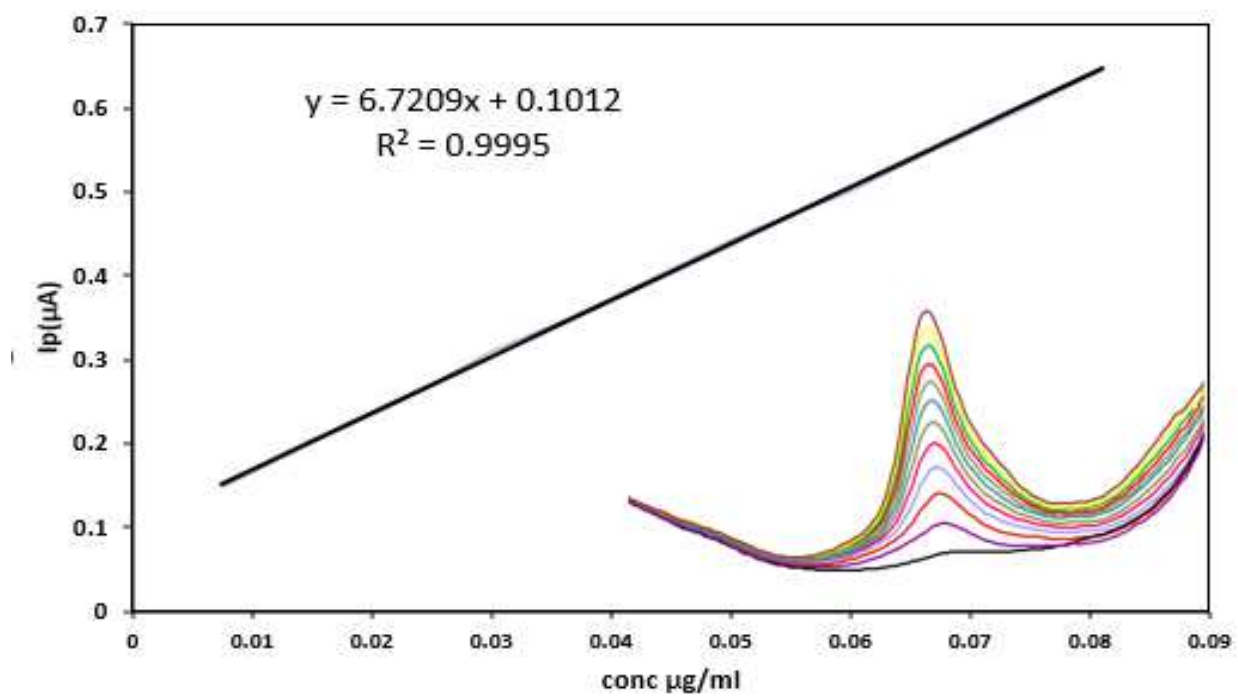


Figure 8. Calibration curve of TDZ using Nano ZnO-CPE in 0.04 M BR electrolyte at pH 2 using DPV method