Full Paper

Electrochemical Approach for Determination of Tertahydrozoline HCl using Gold Nanoparticles Electrode

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Abstract- The electrochemical behavior of tetrahydrozoline (THZ) was explored by cyclic voltammetry and differential pulse voltammetry. A well-defined peak at 915 mV using gold nanomodified electrode (AuNPts/CP) in presence of sodium dodecyl sulphate (SDS) was detected. Different buffers, pH and scan rates on oxidation peak current were studied. Linear calibration curve was achieved in the range of 1.99×10^{-5} to 7.79×10^{-5} M with a limit of detection (LOD) 5.54×10^{-6} M and limit of quantification (LOQ) 1.69×10^{-5} M, respectively. The suggested method was successfully utilized to the eye drops dosage form and urine sample with percentage recovery, 98.86%±0.77, and 101.13±0.62, respectively.

Keywords- Tetrahydrozoline, Voltammetry, Gold nanoparticles, Carbon paste, Surfactant, electroanalysis

1. INTRODUCTION

Tetrahydrozoline (*RS*)-2-(1,2,3,4-tetrahydronaphthalen-1-yl)-4,5-dihydro-1H-imidazole as HCl salt. (Fig. 1)

Scheme 1. Chemical structure of THZ

THZ is an imidazole derivative which stimulates alpha-adrenergic receptors causing vasoconstriction for ophthalmic and nasal congestion treatment [1].

Looking for literature review for THZ analysis, titration with perchloric acid for bulk analysis was reported [2].UV/VIS spectrophotometry for THZ analysis in bulk , nasal and ophthalmic solutions [3]. High-performance liquid chromatography (HPLC) [4,5] and reversed-phase ion pair chromatography [6]. It is was also estimated simultaneously with other substances including;

Antazoline [7], fluorometholone [8], naphazoline [9], ofloxacin and prednisolone acetate [10], in combination of its decomposition products [11], capillary electrophoresis[12], gas chromatography [13] , and charge transfer complex method [14].

The proposed method came out on top since no study was employed for THZ estimation electrochemically.

The novelty of the suggested work was the ability of the AuNPts/CP/SDS electrode to display the differential pulse voltammetry (DVP) for quantification of the cited drug. Taking into our consideration the usefulness of the electrochemical analysis, the work is advantageous on account of short time of analysis, low-cost and eco-friendly [15,16]. The characteristics of CP for being cheap, ease of utilization and low ohmic resistance payed high attention to its application for drug analysis [17]. The nanomaterials such as gold particles boosted a good electron transfer with electro catalytic action [18,19].

Recently, some of toxicity cases was detected as a result of high concentration of THZ in blood and urine. Consequently, application of our study to real sample was crucial. The goal of this work was to provide rapid, simple and eco-friendly electrochemical method for quantification of THZ in bulk, eye drops and in spiked urine.

2. EXPERIMENTAL

2.1. Apparatus

Metrohm analyzer competence system 797VA software 1.0 (Switzerland) was utilized. The utilized electrodes were CPE/or (AuNPts/CPE) as a working electrode, Ag/AgCl (3 M KCl) as a reference electrode and a platinum electrode as an auxiliary one. The pH meter Jenway 3510 with a combined glass electrode was used for pH measurements. Deionized water was supplied

by the Hamilton-Aqua-Metric system. The proposed method was performed at an ambient temperature of 25±0.1 °C. Scanning electron microscopy (SEM) measurements were carried out using a JSM-6700F scanning electron microscope (Japan Electro Company).

2.2. Reagents and Chemicals.

All chemicals and reagents were of analytical grade.

- Britton-Robinson buffer (BR) solutions were prepared 0.04 M by mixing 0.04 M of phosphoric acid, 0.04 M acetic acid, and 0.04 M boric acid. Suitable amount of 0.20 M NaOH was added to get the pH from 2.00-12.00 [20].
- Anionic surfactants, heptane sulfonic acid (HSA), octane sulfonic acid (OSA), Sodium dodecyl sulphate (SDS), cationic surfactant, cetyltrimethylammonium bromide (CTAB), and non-ionic surfactant Triton X were provided from Alfa Aesar, Germany.
- THZ HCl reference standard was supplied from United States Pharmacopeia (Rockville, MD, USA), its purity was 100%.
- Visine® eye drop, batch no. IIB2P00 labelled to contain 0.05% Tetrahydrozoline HCl provided by Johnson & Johnson.
- Graphite powder ultrapure with particle dimension 20 μm, tetrachloroaurate, and paraffin oil were provided from Alfa Aesar, Germany.
- Boric acid, phosphoric acid, acetic acid, sodium hydroxide, potassium nitrate, Potassium biphthalate and Ammonium acetate were provided by El-Nasr pharmaceutical company, Cairo, Egypt.
- Deionized water.

2.3. Working Electrodes

(a) The CPE was made by mixing-up (0.5 g) of ultra-pure graphite powder with (0.3 mL) of paraffin oil. The obtained paste was packed into an insulin syringe that represent the body of the working electrode [21]. Then, polishing the surface of the electrode by using filter paper to get the shiny look.

(b) Modified CPE with gold nanoparticles was fabricated by immersing the CPE into a 6 mM hydrogen tetrachloroaurate $HAuCl_4$ solution containing 0.1 N KNO_3 [22].
Potential of -0.4 V was applied for 400 s to get the prepared electrode and left to dry for 20 minutes before starting the measurements.

2.4. Standard solutions

Accurately weight of 0.05 g of THZ HCl was dissolved in deionized water in a 25 mL volumetric flask to get (1.00×10^{-2} M) stock solution. For preparing working solution, 3 ml of

stock solution was transferred to 10 mL volumetric flask in deionized water to get solution of (3.00×10^{-3} M). Several aliquots from the working solution were taken and added to the voltammetric cell. A stock solution was stable in the refrigerator at 4 °C for 10 days.

2.5. Suggested analytical procedure

2.5.1. Calibration curve of THZ

The voltammetric behavior of THZ HCl was studied through cyclic voltammograms using the AuNPts/CPE electrode. An appropriate aliquot of THZ HCl working standard was placed in voltammetric cell and 5 µl of 5.00×10^{-3} M SDS solution were added to the electrolytic cell containing 15 mL of BR buffer of pH 10. The solution was stirred with a pre-concentration time 10 s and applied potential (E_{app}) -300 mV then allowed to stop for 5 seconds. The cyclic voltammogram was established at a scan rate of 100 mVs^{-1}.

2.6. Instrumentation

2.6.1. Apparatus

All voltammetric measurements were performed using the Metrohm analyzer competence system 797VA software 1.0 (Switzerland). The utilized electrodes were CPE /or (AuNPts/CPE) as a working electrode, Ag/AgCl (3 M KCl) as a reference electrode and a platinum electrode as an auxiliary one. The pH meter Jenway 3510 with a combined glass electrode was used for pH measurements. Deionized water was supplied by the Hamilton-Aqua-Metric system. The proposed method was performed at an ambient temperature of 25±0.1 °C. Scanning electron microscopy (SEM) measurements were carried out using a JSM-6700F scanning electron microscope (Japan Electro Company).

2.7. Analysis of eye drops

Different aliquots of 0.05% Visine® eye drop was introduced into the electrochemical cell and the previous method was applied as described before.

2.8. Application to spiked human urine

The diluted urine sample was prepared by mixing 10 mL urine and 90 mL BR buffer at pH 10. Different aliquots of THZ working standard were added to the cell containing 10 mL of the diluted urine, 15 mL BR buffer and 5 µl of 5.00×10^{-3} M SDS surfactant solution. The voltammograms were established.

3. RESULTS AND DISCUSSION

3.1. Voltammetric behavior of THZ

The behavior of THZ solution 4.14×10^{-5} M in BR buffer of pH 10 at AuNPts/CPE was depicted by cyclic voltammetry (CV). Cyclic voltammogram of THZ showed one anodic oxidative peak with current $I_p=2.00\times10^{-7}$ A at 0.915 V. No reverse peak was detected showed irreversible reaction (Figure 1).
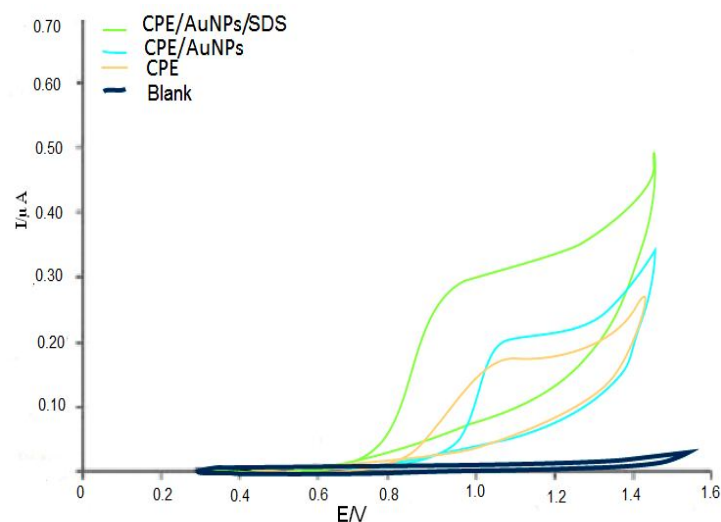

Fig. 1. Cyclic voltammogram of 4.14×10^{-5} THZ solution in BR buffer pH 10 at different electrodes (CPE and AuNPts/CPE)
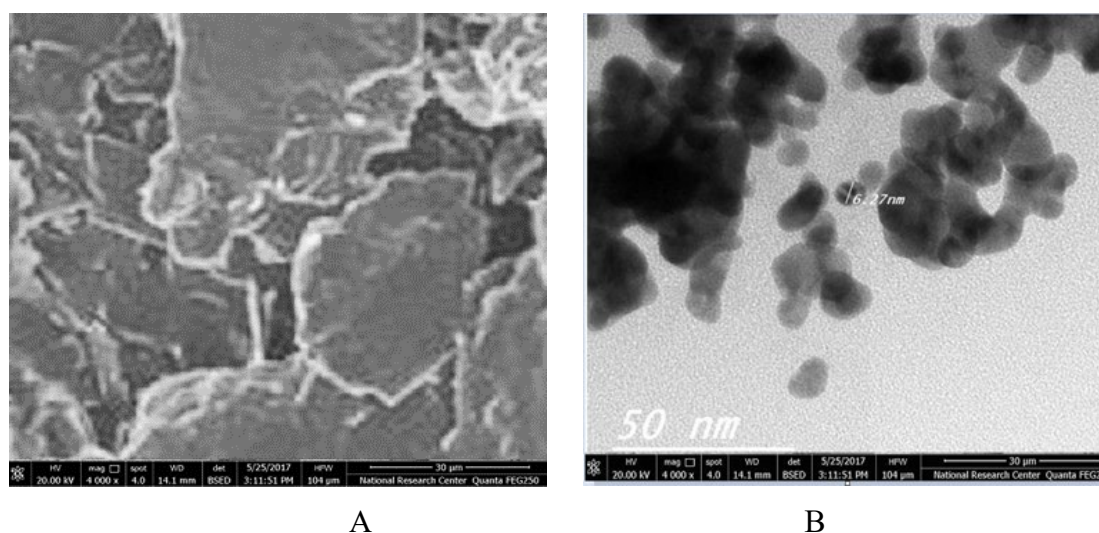

A   B

Fig. 2. SEM of CPE (A) and, TEM of AuNPts/CPE (B) images

3.2. Characterization of CPE and AuNPts/CP electrodes

The images of SEM of CPE (A) and AuNPts/CPE (B) employed a difference in the surface of the CPE was flakes in shape layers (Figure 2A). The AuNPts showed spherical shape as a result of gold particles deposition with large conducting surface area. (Figure 2B). For that, AuNPts/CPE was the best choice as working electrode for the measurements.

3.3. The studied experimental conditions

3.3.1. Buffer type and pH

The cyclic measurements of different buffer types of THZ solution 4.14×10^{-5} M were examined. The highest current value was achieved with BR buffer (Figure 3A). By applying the general procedure in BR buffer of pH range from 3.00 and 12.00, the highest peak current value was achieved at pH 10 then the current started to decrease (Figure 3B).
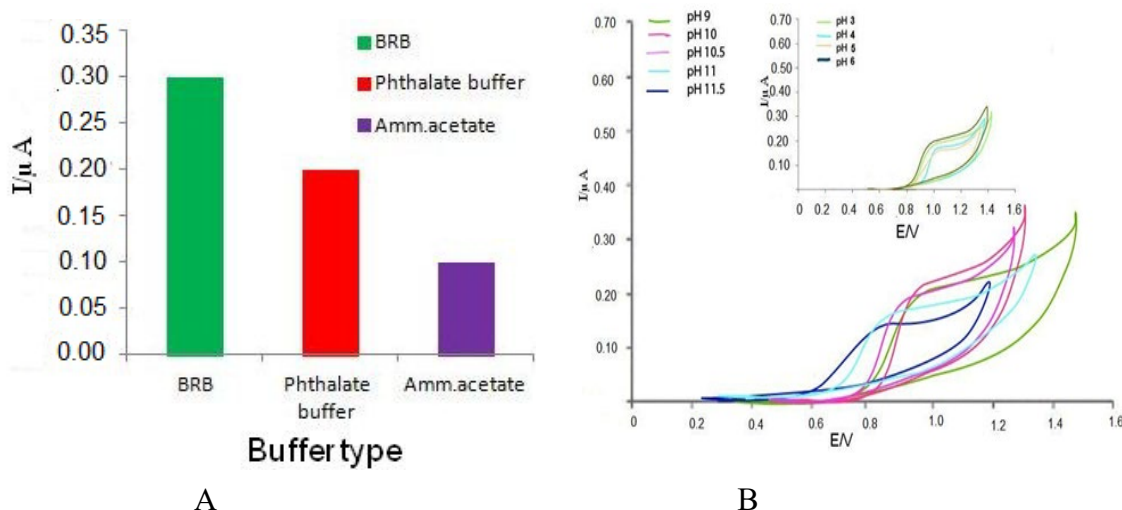

Fig. 3. (A) The effect of buffer types on the current of THZ at pH 10, (B) The effect of pH on cyclic voltammograms 4.14×10^{-5} M of THZ solution at AuNPts/CPE

It was found that as the pH increased the potential shifted to the negative side. The oxidation peak potential was dependant on protonation/deprotonation that controlled the charge transfer process [23]. E_p=K (0.059y/n) pH [24], as y was the H^+ number ions and the electrons number was n. Linear relationship between pH and peak potential (E_p) was obtained for THZ with regression equations of E (V)=0.042pH+1.30, and (r^2)=0.9895 (Figure 4.)

The slope found to be 0.042 concluded that one electron and one proton participated in the reaction at the surface of AuNPts/CPE.
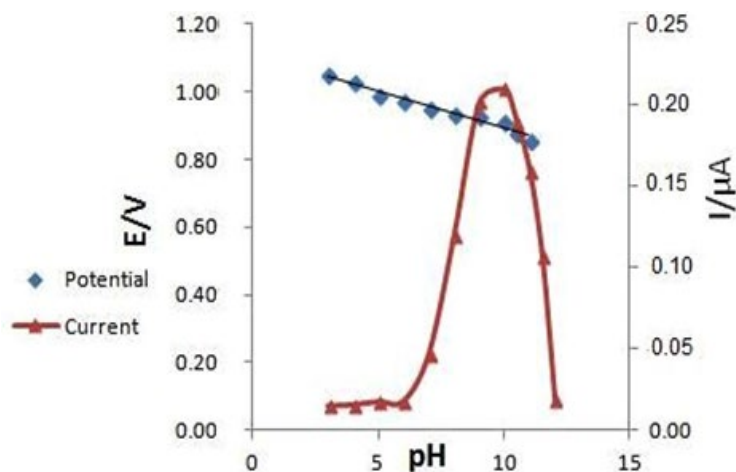
Fig. 4. Plot of the relationship between peak potential and current as a function of pH by cyclic voltammetry of 4.14×10^{-5} M THZ at the of AuNPts/CPE

3.4. Effect of different surfactants

The electrochemical response of THZ at the AuNPts/CPE electrode surface was greatly affected by the surfactant [25]. Different types of surfactant varying in length of the hydrocarbon chain and charge were added to the voltammetric cell. A non-ionic surfactant (Triton X), an anionic (SDS), and cationic surfactant (CTAB) of the same concentration (5.00×10^{-3} M) were added to the electrolytic cell with 4.14×10^{-5} M THZ in BR buffer of pH 10 at the AuNPts/CPE.
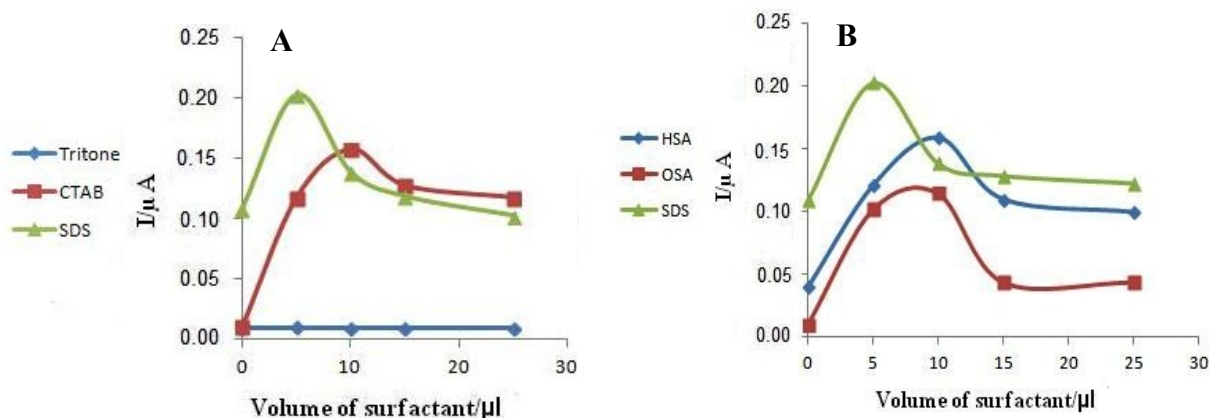

Fig. 5. (A)The influence of surfactants types on the current of THZ solution 4.14×10^{-5} M in 0.04 M BR buffer. (B) The effect of volume of different anionic surfactants on peak current of THZ solution 4.14×10^{-5} M in 0.04 M BR buffer

By applying the CV method using different volumes of surfactant (5.00×10^{-3} M), the voltammograms showed an increase of peak current when adding the surfactant to definite limit (5 µl) as shown in Figure 5A. Any increase of surfactant volume more than 5 µl caused a

decrease in electrochemical response. SDS showed highest current compared to Triton X and CTAB. The adsorption of micelle that is formed on the surface of the working electrode caused a decrease of current with volume of surfactant more than 5 μl [26]. The highest current of THZ was achieved with SDS when using the same concentration of HSA, SDS and OSA $(5.00 \times 10^{-3}$ M) (Figure 5B). The reason for that was the aggregation of the positively charged drug with nitrogen atom of the primary amine at pH 10 with SDS carrying negatively charged groups. The hydrocarbon chain length of surfactant extensively affected the critical micelle concentration (CMC) [27] .

3.5. The scan rate effect

Studying the voltammetric response of THZ 4.14×10^{-5} M at the AuNPts/CPE in BR buffer pH 10 in the scan rate range 50 to 200 mV s^{-1} displayed linearity between logarithm of the peak current versus logarithm of the scan rate covering the range 60-180 mVs^{-1} The linear regression equation found to be;

Log I=0.722 log υ-1.12 (r^2=0.9985), as shown in (Figure 6 A,B)

The value of the slope was 0.722. It was concluded that the electron transfer is adsorption-controlled reaction [28].

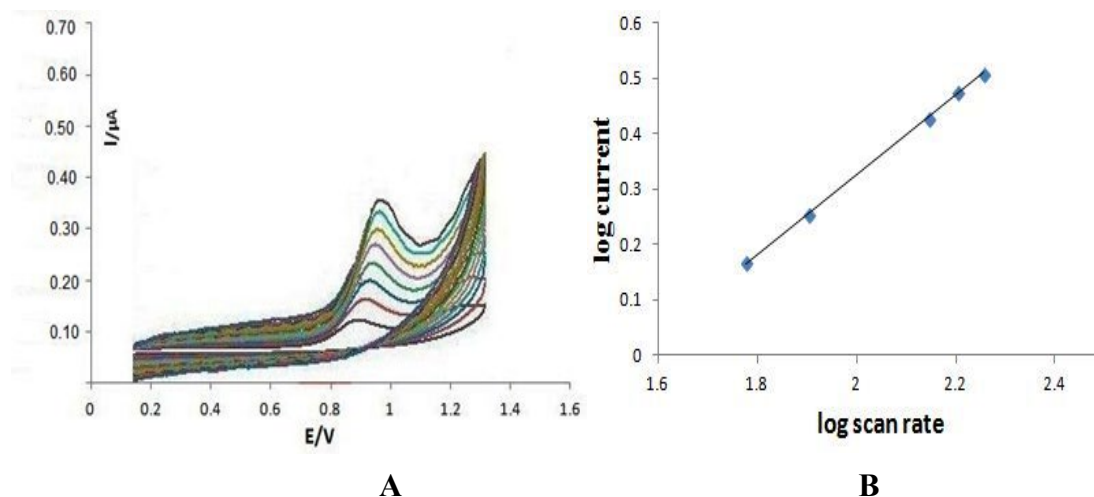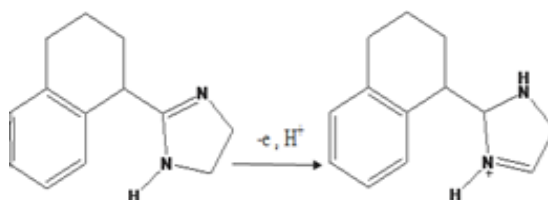**A** **B**

Fig. 6. (A) Cyclic voltammograms of THZ solution 4.14×10^{-5} M in 0.04 M BR buffer solution pH 10 at the AuNPts/CPE at different scan rates; (B) Plot of logarithm of the peak current *vs.* the logarithm of the scan rate

Laviron equation explained the number of electrons involved in the reaction [29] as following;

$E_p = E° + (2.303RT/αnF) [\log RTK°/αnF + \log \upsilon]$

As α is the electron transfer coefficient, n is the number of electrons, T is the temperature (298 K), R is the gas constant (8.314 JKmol^{-1}), and F is the Faraday constant (96.485 Cmol^{-1}). The α n calculated from the slope 0.722 and α (electron transfer coefficient) was 0.5. Therefore, the value of n≈1. (Scheme 2).

Scheme 2. The proposed oxidation mechanism of THZ

3.6. Validation of the suggested method.

The guidelines stated by ICH Q2 (R1) was applied for validation of the suggested DPV method for estimation of THZ [31].

3.7. Linearity and range

There was a dependency of current upon the concentration of the drug under the optimum conditions covering the concentration range of 1.99×10^{-5} M to 7.79×10^{-5} M (Figure 7).

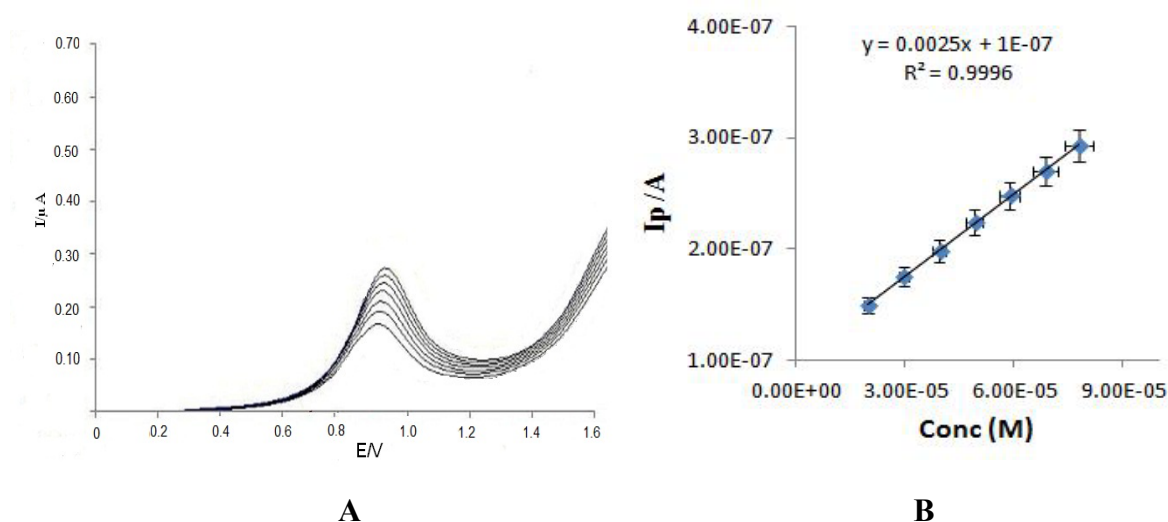A   B

Fig. 7. (A) DPV voltammograms of THZ at the AuNpts/CPE/HSA in 0.04 M BR buffer solution pH 10; (B) Plots of linearity of various concentrations of THZ concentrations and the corresponding current

The regression equation was computed by;

$I_p = 0.0025C + 0.0000001$ (r = 0.9996)

As I_p is the anodic peak current, C is the concentration of THZ in M, and r is the correlation coefficient (Table 1).

3.8. Limits of detection and quantification

Linearity range established before was used for determination of limit of detection (LOD) and limit of quantification (LOQ) that calculated by the formulas;

$$LOQ=10\sigma/b$$
$$LOD=3.3\sigma/b$$

As $\sigma=S_{y/x}$ residual standard deviation of the response and b=slope of the linearity. Values of LOD and LOQ were calculated to be 5.54×10^{-6} M and 1.69×10^{-5} M, respectively as given in Table 1 All calculations were done by Excel program.

Table 1. Regression parameters of DPV method for THZ assay in bulk

Parameters	THZ. HCl
Linearity range (M)	1.99×10^{-5} to 7.79×10^{-5}
Slope (b)	0.0025
Intercept	0.0000001
Correlation coefficient (r)	0.9996
LOD (M)	5.54×10^{-6}
LOQ (M)	1.69×10^{-5}
Sy/x	1.59×10^{-9}
SE of the slope	3.09×10^{-5}
SE of the intercept	1.63×10^{-9}

LOD=limit of detection, LOQ=limit of quantification, SD=standard deviation, Sy/x=standard deviation of residual, SE=standard error

3.9. Repeatability and intermediate precision.

The precision was assessed by applying triplicate analyses of 3 different concentrations in the same day over the linearity range. The inter-day variation was performed by measuring 3 different concentrations for 3 successive days. The obtained results demonstrated good reproducibility of the suggested method over the linearity range (Table 2).

Table 2. Repeatability and intermediate precision of the suggested DPV method for the determination of THZ in bulk

Concentration taken	Intra-day precision				Inter-day precision			
	Mean Concentration found[*]	Recovery (%)	SD	RSD (%)	Mean Concentration found[*]	Recovery (%)	SD	RSD (%)
2.97×10^{-5}	2.96×10^{-5}	99.56	0.55	0.55	3.00×10^{-5}	101.01	0.99	0.98
3.95×10^{-5}	3.92×10^{-5}	99.20	0.44	0.44	3.91×10^{-5}	99.27	0.65	0.65
4.92×10^{-5}	4.87×10^{-5}	99.96	0.47	0.47	4.89×10^{-5}	99.61	0.55	0.56

* Average of three determinations, n =3. SD = standard deviation, RSD = relative standard deviation

3.10. Robustness

The ability of analytical method to remain unaffected upon minor changes in experimental parameters was studied. In order to demonstrate the robustness of the method, the constancy of the anodic peak current with minor changes in experimental parameters such as electrolyte pH 10 ± 0.20 was investigated. The minor changes did not affect the current intensity as shown in (Table 3).

Table 3. Robustness of the suggested DPV method

Parameters	THZ HCl	
%Recovery[*]		%RSD
pH 9.80	98.97	0.70
pH 10.00	99.69	0.68
pH 10.20	98.50	0.19

*Average of three determinations, n =3. RSD = relative standard deviation

3.11. Standard addition technique application

Different concentrations of standard solution of THZ were added to constant concentration of the dosage form visine drop. The resulted mean percent recovery ±RSD% found on average of three replicate measurements 98.83 ± 0.64 displayed no important interference from these excipients (Table 4).

Table 4. Standard addition technique application

	Concentration taken (M)	Concentration added (M)	Concentration found (M)	%Recovery[*]	SD
Visine®eye drops 0.5 mg/ml	2.96×10^{-5}	1.99×10^{-5}	1.96×10^{-5}	98.65	0.50
		2.97×10^{-5}	2.96×10^{-5}	99.54	0.51
		3.95×10^{-5}	3.88×10^{-5}	98.29	0.25
Mean±SD					98.83±0.64
RSD%					0.65%

*Average of three determinations, n =3. SD = standard deviation, RSD = relative standard deviation

3.12. Eye drops application

DPV method was applied for quantitative determination of THZ in dosage from. By comparing the statistical data obtained from applying DPV method for THZ determination with the reported method [4], it was proved that no significant difference between the two methods using the calculated t an F values (Table 5).

Table 5. Comparing the statistical data of the suggested method with the reported one [4]

Visine®eye drops 0.5mg/ml	Suggested DPV method				Reported method [4]
	Concentration taken (M)	Concentration found (M)	%Recovery[*]	SD	
	2.48×10^{-5}	2.48×10^{-5}	100.19	0.51	
	2.96×10^{-5}	2.92×10^{-5}	98.50	0.57	
	4.10×10^{-5}	4.04×10^{-5}	98.58	0.22	
	4.90×10^{-5}	4.84×10^{-5}	98.74	0.26	
	5.70×10^{-5}	5.60×10^{-5}	98.24	0.32	
Mean*±RSD%	98.86±0.77				99.92±0.88
Student's t-test					1.88 (2.30)[a]
F-Test					1.33 (6.38)[b]

SD=standard deviation. RSD=standard deviation, Mean* n=5. a,b the values in parenthesis represented the theoretical values of t and F at P=0.05 [31]. *Average of three determinations, n=3

3.13. Application in spiked urine sample.

The proposed method was applied to urine sample. The recovery (%) was calculated with low SD value. It confirmed the suitability of DPV application of THZ in urine sample as shown in (Table 6).

Table 6. Determination of THZ in the urine sample by the proposed DPV

Suggested DPV method				
Urine sample	Concentration taken (M)	Concentration found (M)	Recovery (%)*	SD
	2.77×10^{-5}	2.79×10^{-5}	100.72	0.38
	3.36×10^{-5}	3.38×10^{-5}	100.59	0.45
	6.27×10^{-5}	6.33×10^{-5}	101.76	0.56
	6.46×10^{-5}	6.58×10^{-5}	101.85	0.16
	7.22×10^{-5}	7.33×10^{-5}	101.52	0.21
Mean*±RSD%	101.13±0. 62			

SD=standard deviation. Mean* n=5
*Average of three determinations, n=3

4. CONCLUSION

The electrochemical proposed study investigated the behavior of THZ at AuNPts/CPE with displayed defined irreversible anodic response. The results stated linearity over the range 1.99×10^{-5} to 7.79×10^{-5} M. The oxidation peak showed significant current increase when SDS was added to the electrochemical cell. It is worth mentioned that there was no reported method for THZ determination electrochemically. The proposed DPV approach described the

oxidation activity of THZ at AuNPts/CPE. Moreover, the fabricated AuNPts/CPE provided high sensitivity and eco-friendly approach for estimation of THZ. Furthermore, a good voltammetric response was achieved by employed the suggested DPV method to the eye drops dosage form and urine analysis without matrix interference.

REFERENCES

[1] J. E. F. Reynolds. Martindale, the Extra Pharmacopoeia, 30th Edition, Pharmaceutical Press, London (1993).

[2] British Pharmacopeia, London, UK (2010).

[3] United States Pharmacopeia, National Formulary 26, Rockville, MD (2011).

[4] F. Al-Rimawi, W. Zareer, S. Rabie, and M. Quod, J. Pharma. Anal. 2 (2012) 67.

[5] G. Andermann, and A. Richard, J. Chromatogr. A 298 (1984) 189.

[6] G. Puglisi, S. Sciuto, and R. Chillemi, J. Chromatogr. A 369 (1986) 165.

[7] P. Rojsitthisak, W. Wichitnithad, and O. Pipitharom, J. Pharm. Sci. Technol. 59 (2005) 332.

[8] T. G. Altuntas, F. Korkmaz, and D. Nebioglu, Pharmazie 55 (2000) 49.

[9] J. Bauer, and S. Krogh, J. Pharm. Sci. 72 (2006) 1347.

[10] M. S. Ali, M. Ghori, and A. Saeed, J. Chromatogr. Sci. 40 (2002) 429.

[11] B. Stanisz, Kinet. Catal. Lett. 74 (2004) 135.

[12] M. Gumustas, U. Alshana, N. Ertas, N. G. Goger, S. A. Ozkan, and B. Uslu, J. Pharm. Biomed. Anal. 124 (2016) 390.

[13] J. Peat, and U. Garg, Method. Mol. Biol. (Clifton, N. J.) 603 (2010) 501.

[14] G. G. Mohamed, M. S. Rizk, and E. Y. Z. Frag, Iran J. Pharm. Res. 14 (2015) 701.

[15] F. Sayın, and S. Kır, J. Pharm. Biomed. Anal. 25 (2001) 153.

[16] S. A. Özkan, B. Uslu, and P. Zuman, Anal. Chim. Acta 457 (2002) 265.

[17] B. J. Sanghavi, and A. K. Srivastava, Electrochim. Acta 56 (2011) 4188.

[18] H. M. Ahmed, M. A. Mohamed, and W. M. Salem, Anal. Methods 7 (2015) 581.

[19] N. F. Atta, A. Galal, A. A. Wassel, and A. H. Ibrahim, Int. J. Electrochem. Sci. 7 (2012) 10501.

[20] H. T. S. Britton, and R. A. Robinson, J. Chem. Soc. (Resumed) (1931).

[21] A. K. Attia, M. M. Abd-Elmoety, A. M. Badawy, A. E. Abd Elaleemand, and S. G. Abd-Elhamid , Anal. Bioanal. Chem 1 (2014) 128.

[22] N. F. Atta, A. Galal, and S. M. Azab, Int. J. Electrochem. Sci. 6 (2011) 5082.

[23] I. Streeter, G. G. Wildgoose, L. Shao, and R. G. Compton, Sens. Actuators B 133 (2008) 462.

[24] N. F. Atta, S. A. Darwish, S. E. Khalil, and A. Galal, Talanta 72 (2007) 1438.

[25] F. Scholz, Books on Fundamental Electrochemistry and Electroanalytical Techniques, Electro. Anal. Methods, Springer (2010) 343.

[26] A. O. B. P. Industry, ABPI Compendium of Data Sheets and Summaries of Product Characteristics: With The Code of Practice for the Pharmaceutical Industry. 1999-2000, Datapharm Publications Limited (1999).

[27] J. F. Rusling, Coll. Surfaces A 123 (1997) 81.

[28] B. Peterson, and C. J. Marzzacco, The Effect of Hydrocarbon Chain Length on the Critical Micelle Concentration of Cationic Surfactants: An Undergraduate Physical Chemistry Experiment (2007).

[29] D. Skoog, D. West, and F. Holler, Fundamentals of Analytical Chemistry Saunders College, Philadelphia, Estados Unidos (1996).

[30] E. Laviron, and J. Electro. Anal. Chemi. Inter. Electro. 101 (1979) 19.

[31] IHT Guideline, Validation of analytical procedures: text and methodology Q2 (R1), International Conference on Harmonization, Geneva, Switzerland (2005).

[32] R. Caulcutt, and R. Boddy, Statistics for analytical chemists, Chapman & Hall (1983).