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ELECTROCHEMICAL BEHAVIOUR OF TROMANTADINE HYDROCHLORIDE AT DIFFERENT ELECTRODES AND ITS ANALYTICAL APPLICATIONS

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Abstract:
A simple, sensitive, rapid and precise method was developed using voltammetric techniques namely; cyclic voltammetry, differential pulse and square wave voltammetry for the determination of Tromantadine Hydrochloride (TH) at three different working electrodes namely; glassy carbon (GCE), carbon paste (CPE) and pencil graphite (PGE) electrodes. Firstly, we began with cyclic voltammetric analyses to optimize the voltammetric conditions. TH cyclic voltammogram revealed a well-defined irreversible anodic peak at about 0.751 V, 0.692 V and 0.784 V at GCE, CPE and PGE, respectively. The oxidation process was shown to be irreversible diffusion-controlled for GCE and PGE and adsorptive stripping for CPE. Based on this study, a sensitive quantitative method is recommended for the determination of TH as a pure raw material and in its pharmaceutical formula. The proposed method is validated and compared with the results obtained from the reported valid method. It revealed good accuracy and reproducible results.

Keywords: *Tromantadine Hydrochloride; Voltammetry; Electrochemical Determination; Oxidation; Adsorptive Stripping; Glassy Carbon Electrode; Carbon Paste Electrode; Pencil Graphite Electrode.*

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INTRODUCTION:

Tromantadine Hydrochloride (TH); N-((3s, 5s, 7s)-adamantan-1-yl)-2-(2-(dimethyl amino) ethoxy) acetamide hydrochloride (Fig. 1) is a non-official drug used to treat herpes simplex virus. Antiviral drugs treat viral infections , they do not destroy their target pathogen; instead they retard and obstruct their development [1-5].

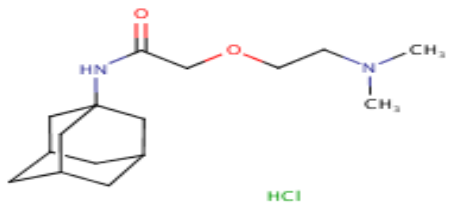

Fig. 1. Chemical structure of Tromantadine Hydrochloride.

Antiviral drugs are a class of antimicrobials, a larger group which also includes antibiotics , antifungals and antiviral drugs based on monoclonal antibodies [6]. Most antivirals considered relatively harmless to the host, and therefore can used to treat infections. They should be differentiated from vermicides, which are not considered as medication but only deactivate or destroy virus particles, either inside or outside human body [7]

TH is available in a topical gel under trade name Viru-Merz®. Its behavior is like acyclovir. Tromantadine obstructs the early and late stages in the virus replication cycle. It alters the glycoproteins of the host cells, therefore hindering the absorption of the virus. It hinders penetration of the virus and also prevents uncoating of the virions [3].

It has been proved that electrochemical analyses are undoubtedly incisive for the determination of organic molecules such as drugs and other molecules present in pharmaceutical drug products [8-12]. The progress in electrochemical techniques in the territory of examination and determination of drugs because of their frankness, sensitivity and rapidness straightaway compared with different techniques. The utilization of carbon-based electrodes, especially glassy carbon electrode, for electrochemical measurements has grown in the recent years due to their suitability and appropriateness for the determination of substances that undergo redox reactions, which is very important in the field of clinical and pharmaceutical analysis. Redox properties of drugs can tell in advance its metabolic fate or pharmaceutical activity [13, 14].

To date, reviewing the literature showed a report [1, 15] which discusses the metabolism of TH, briefly described an analytical method using GC–MS with electron ionization or chemical ionization mode of detection. This procedure used for metabolite identification and not for quantitation. However, to our knowledge, there is no attempt has been made for the determination of TH. In our present study, we found a unique method to study the voltammetric behavior of Tromantadine Hydrochloride using cyclic, differential pulse and square wave voltammetric techniques.

The aim of this study is to elaborate optimum experimental conditions to investigate the voltammetric behavior and possible oxidation mechanism of Tromantadine Hydrochloride at different electrodes using cyclic voltammetry technique and determine its concentration in its pure raw material and in its pharmaceutical formulation using differential pulse and square wave voltammetric techniques.

EXPERIMENTAL:

Apparatus

Voltammetric measurement obtained using the electrochemical analyzer computrance system with 797VA computrance software (1.0) from Met Rohm, Switzerland. A three-electrode cell used. The working electrodes used in this study are three different types of electrodes such as HB (Rot ring®) pencil graphite electrode, glassy carbon electrode and a carbon paste electrode. Electrical connection with the working electrodes attained by joining a copper wire to the metallic part of the apparatus fixing the pencil. Ag/AgCl (3M L-1 KCl) used as a reference electrode and platinum wire as a counter electrode. The pH measurements done using digital Jenway 3330 Research pH meter and the glass electrode system was calibrated before and after each series of measurements under the same conditions. Automatic pipettes Socorex Swiss used. All experiments performed at an ambient temperature of 25 °C.

Working Electrodes

(a) Glassy carbon electrode (GCE): mini glassy carbon disk electrode of active area: 2.8 mm, for ELCD 641/656. To discard any contamination and enhance the sensitivity and determination of the voltammetric peaks, the GCE was polished manually [16] with 0.5 mm alumina powder on a flat polishing wipe prior before each electrochemical measurement. Then, it rinsed with methanol and double distilled water, and gently dried with a tissue paper. After rubbing down, the electrode washed entirely with water. After this mechanical treatment, The GCE placed in buffer solution and different voltammograms were registered and displayed until we reached a steady state baseline voltammogram.

(b) Carbon **paste electrode (CPE):** the carbon-paste electrode (CPE) is made from a blend of conducting graphite powder and a pasting liquid [17, 18]. In brief, CPE was prepared by mixing 250 mg of sigma graphite powder and 125mg paraffin oil. The resulted paste was then loaded into the end of an insulin syringe (internal diameter: 3.0mm). External electrical contact set by pushing a copper wire down the syringe.

(c) Pencil graphite electrode (PGE): the preparation of Pencil Graphite Electrode was accomplished as described earlier [19, 20]. In brief, a mechanical pencil Model T 0.5 (Rot ring, Germany) of a total length of 60mm and a diameter of 0.5mm. Electrical connection to the lead attained by sheathing a metallic wire around the metallic part of the pencil. A total of 8 mm of pencil immersed in solution per measurement.

Materials and Reagents

All chemicals used throughout the work were of analytical reagent grade (Merck). They used as such without any purification. The water always deionized or twice distilled. Pure TH (99.9%) in powdered form obtained from PHARCO pharmaceuticals and used without further purification. Dosage form of TH purchased from Global Napi Pharmaceutical Company. The Britton Robinson buffer (B-R) [21] (0.04M) from pH 2-11 were prepared from double distilled water [22].

Standard solutions

A stock standard solution of TH (10^{-3} M) was prepared by dispersing 31.69 mg pure TH in double distilled water and the volume was completed to 100 ml using the same solvent.

Working solution (10^{-4} M)prepared by transferring 10 ml of stock solution into a 100 ml measuring flask and completing to the mark with double distilled water.

General Analytical Procedure

Supporting electrolyte B-R buffer of pH 9.0 (25 ml) was placed in the voltammetric cell and the required volume of standard TH solution was added by micropipette. The solution stirred continuously at 1300 rpm. At the end of accumulation period, the stirring stopped, and after 5.0 sec, rest period permitted the solution to become dormant. The used drug was determined by using DPV and SW methods. Aliquots of the drug solution injected into the electrolytic cell and the procedure repeated. The voltammogram recorded.

Assay procedure for Pharmaceutical dosage form

3.169 g of Viru-Merz® gel [23] was accurately weighed and dissolved in 50 ml chloroform in a separating funnel. Then 25 ml of double distilled water added in the separating funnel and shake vigorously for 5 minutes. By this way, TH extracted in the water layer. Then the water layer separated from the benzene layer and transferred into a 100 ml measuring flask. Again, 25 ml of double distilled water added and shake vigorously for 5 minutes. Then the water layer separated from the chloroform layer and completed to 100 ml with double distilled water to obtain a final concentration of 10^{-3} M (stock solution). We transfer suitable aliquots of the stock solution into a measuring flask whose capacity is 25 ml and complete to the mark with distilled water to prepare different concentrations. The procedure then continued as mentioned above in the General analytical procedure section.

RESULTS AND DISCUSSION:

Cyclic Voltammetric Behavior of TH

The electrochemical behavior of TH was illuminated and explained at the three types of electrodes; GCE, CPE and PGE and cyclic voltammetry carried out. TH showed well-defined oxidation peak at a potential around 690-790 mv with no peak in the reversible scan indicating the irreversibility of the reaction as shown in Fig. 2.

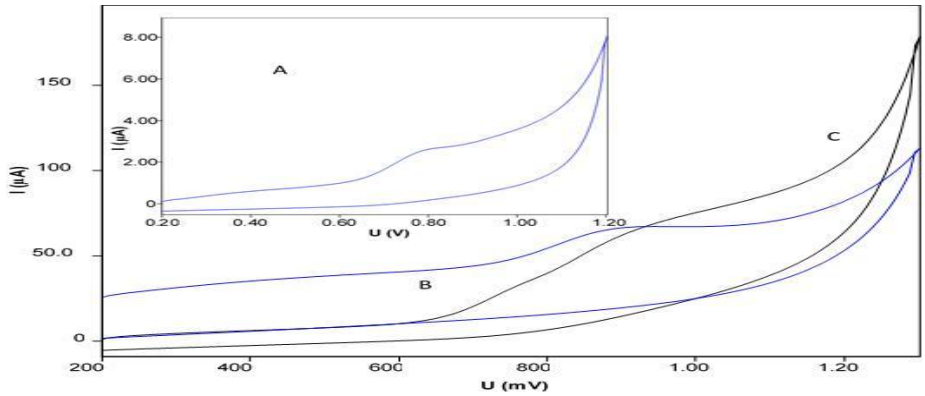

Fig. 2: Cyclic voltammograms of TH using 2 µg/ml at (a) GCE, (b) CPE and (c) PGE, at υ50 mV/s, pre-concentration time = 5s, Eapp = 50 mV.

Effect of pH

The influence of pH on the electro oxidation measured by cyclic voltammograms using Britton–Robinson buffers within the PH range of 2–11. We founded that electrochemical behavior of TH is dependent on the pH value of the aqueous solution and the pH of the solution has a remarkable effect on the peak current and potential of the oxidation of TH as shown in Fig. 3. The maximum current response of TH observed at pH 9.0 at both GCE, PGE and pH 10 at CPE. However, no evident peak observed for TH in the pH range of 2.0–6.0 for the three electrodes. The anodic peak potential (Ep) of TH was found to be dependent on pH and shifted to less positive potential with increasing pH, proposing the participation of protons in the oxidation reaction of TH at GCE, CPE and PGE electrodes. From the plot of Ep vs. PH (Fig. 3), it is obvious that the oxidation peak potential differs linearly with pH and is shifted to more negative by 0.0282, 0.0222 and 0.0249 V/pH for TH for GCE, CPE and PGE electrodes respectively and illustrated by the following regression equations:

Ep (V) = 1.0286 − 0.0282 pH	(r^2 = 0.9685)	
Ep (V) = 0.9588 − 0.0222 pH	(r^2	= 0.9748)
Ep (V) = 1.0723 − 0.0249 pH	(r^2	= 0.968)

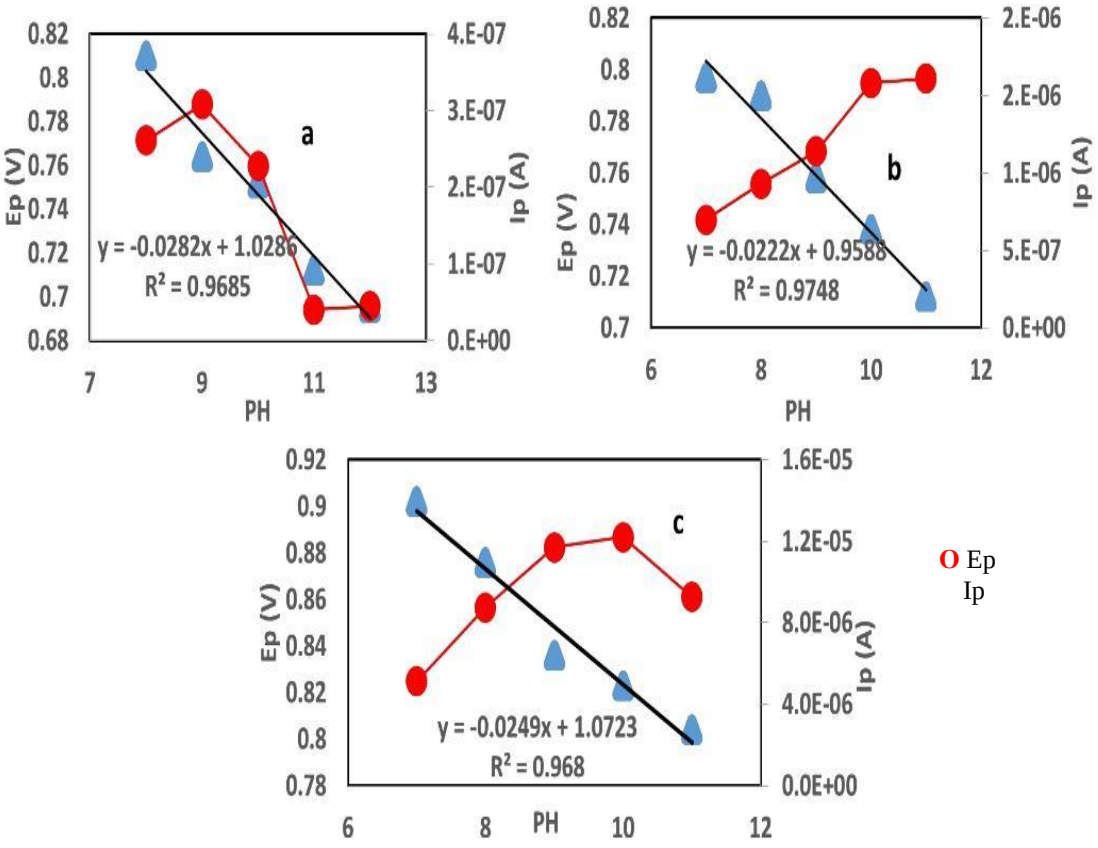

Fig. 3. Effect of pH on peak current and peak potential using (a) 5 mg/ml at GCE, (b) CPE and (c) PGE, at υ 50 mV/s, pre-concentration time = 5s, Eapp = 50 mV.

Effect of scan rate

The influence of scan rate (υ) on the oxidation peak currents of TH studied at GCE, CPE and PGE at the optimum pH for each electrode. The linear variation of logarithm peak current (Ip) with the logarithm of voltage scan rate (υ) for the irreversible electrode reaction was given by the following equation (Randles-sevcik equation) [24]

$$Ip = (2.69 \times 10^5) n^{3/2} A D^{1/2} \upsilon^{1/2} C^*$$

Where n is the number of electrons exchanged during the redox process, A (cm^2) the active area of the working electrode, D ($cm^2\ s^{-1}$) and C * (mole cm^{-3}) the diffusion coefficient and the bulk concentration of the electroactive species; υ is the voltage scan rate ($V\ s^{-1}$).. In the present work, the data plotted as log-log graph. This confirmed the irreversibility of the electrochemical processes with simultaneous increase in the peak current at high scan rate. A good linearity between log Ip and the log scan rate (υ) was obtained from the range of 20 ~ 200 mV/s as shown in Fig. 4. The slope values obtained in case of GCE and PGE are close to the theoretical value (0.5) which demonstrated that the electrode reaction is ideal reaction of the solution species. Thus the oxidation is diffusion [8, 25]. However, in case of CPE, the slope (1.003) is too close to the theoretically expected value (1.0) for an ideal reaction of surface species [25]. Thus the oxidation is adsorption. The application of Randles-sevcik equation is clearly explained in figures 5 and 6.

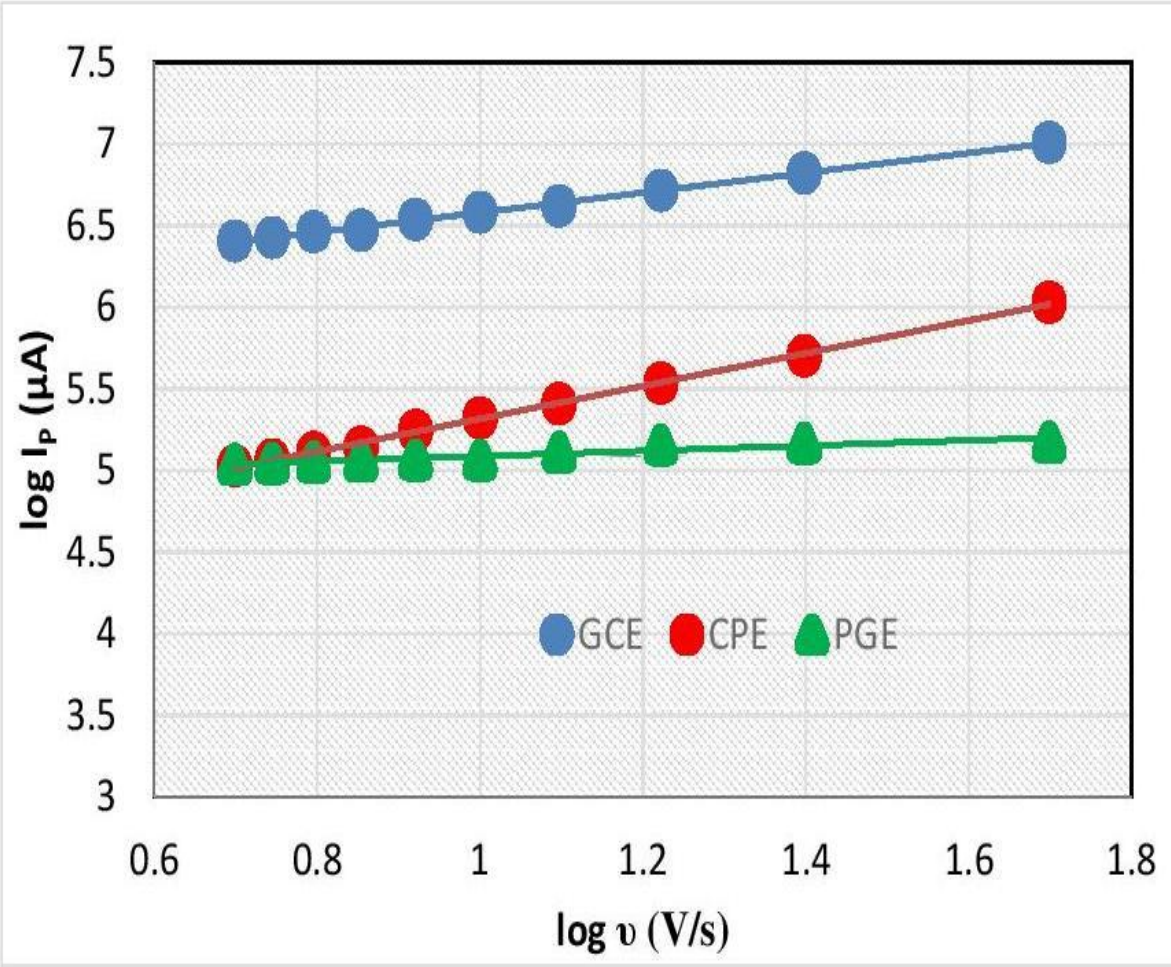

Fig. 4. Shows relation between log I (μA) and log υ (V/s) for oxidation of TH at GCE, CPE and PGE.

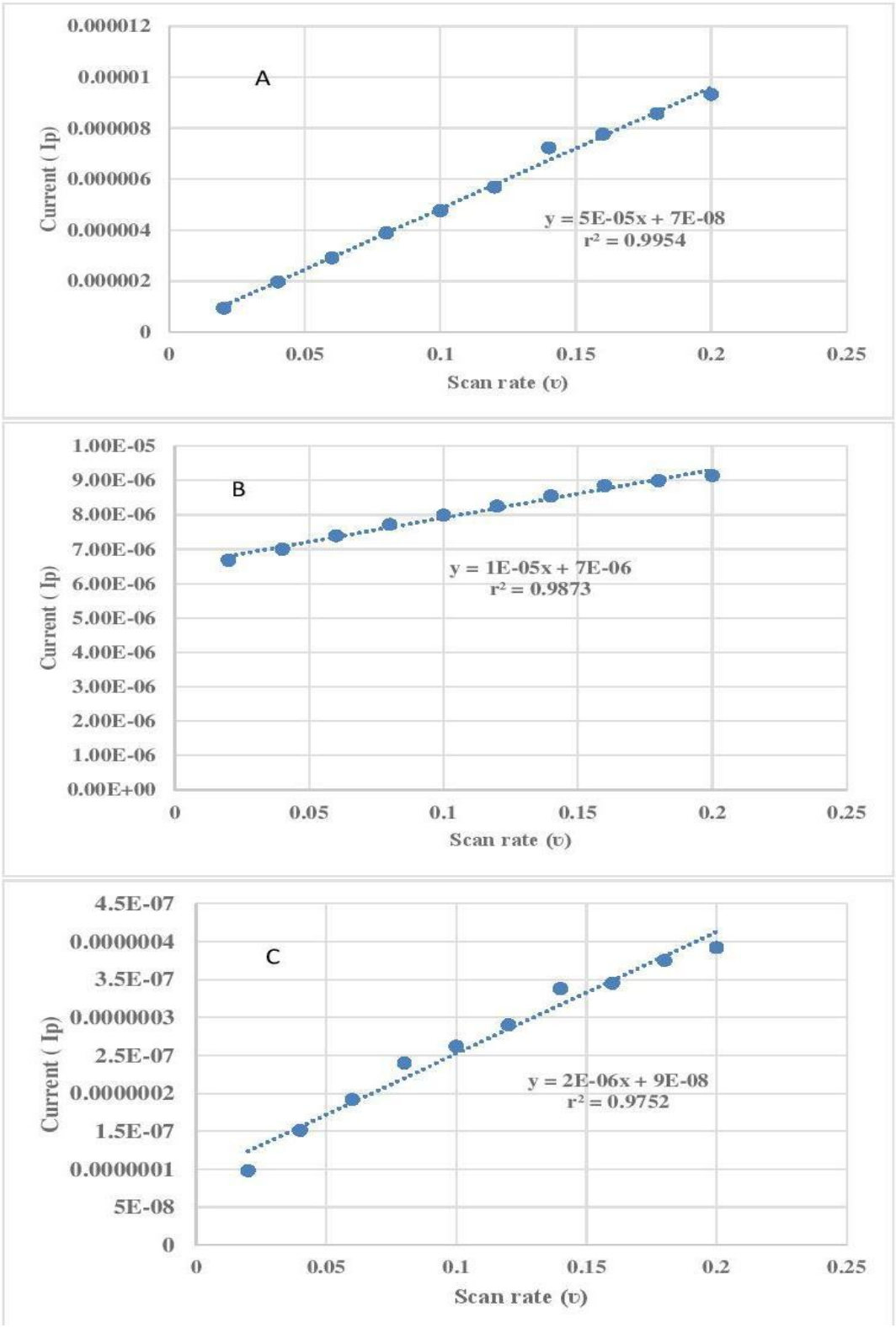

Fig.5. shows relationship between current (Ip) and scan rate (υ) at (A) GCE, (B) CPE and (C) PGE.

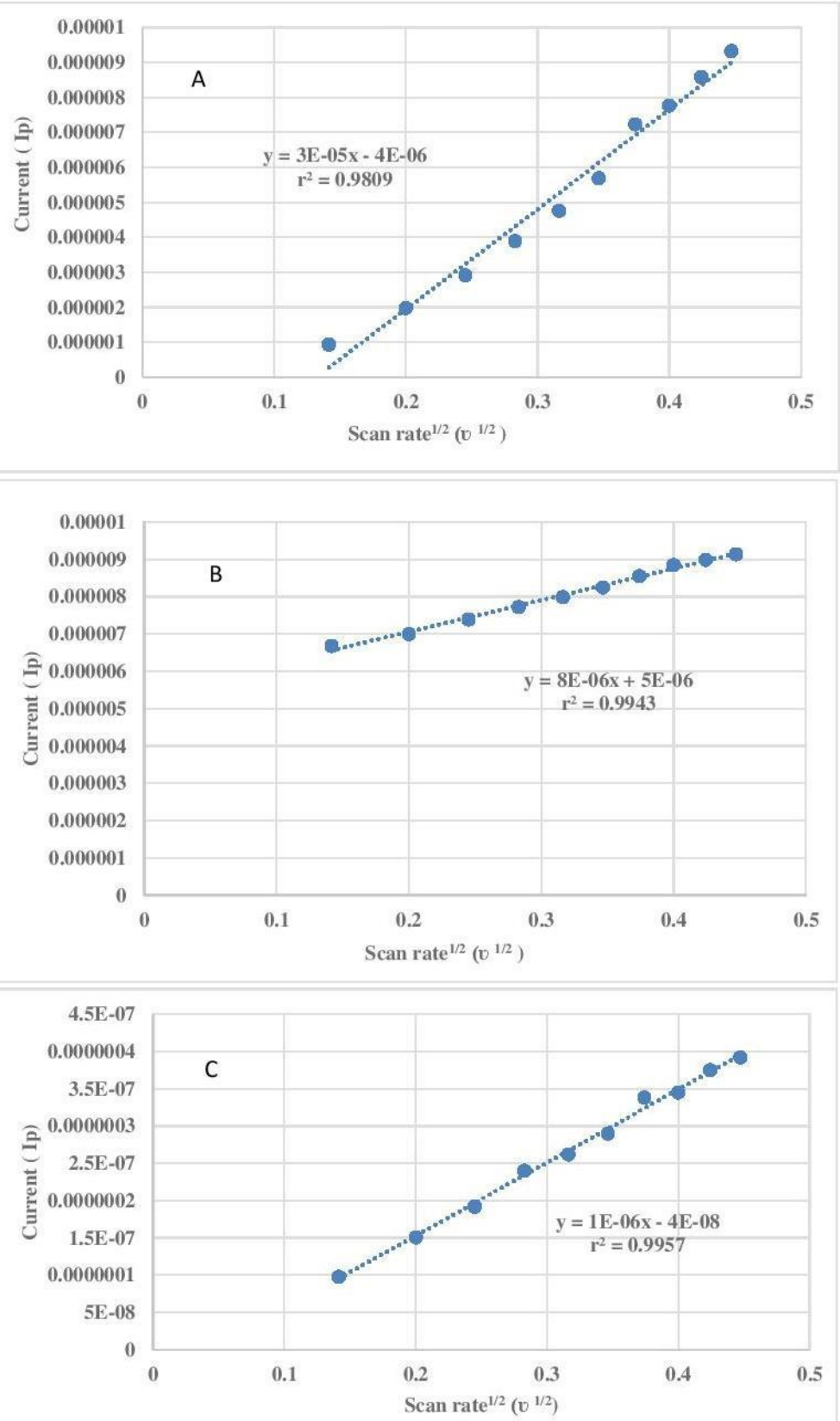

Fig.6. shows relationship between current (Ip) and Scan rate ½ (υ ½) at (A) GCE, (B) CPE and (C) PGE.

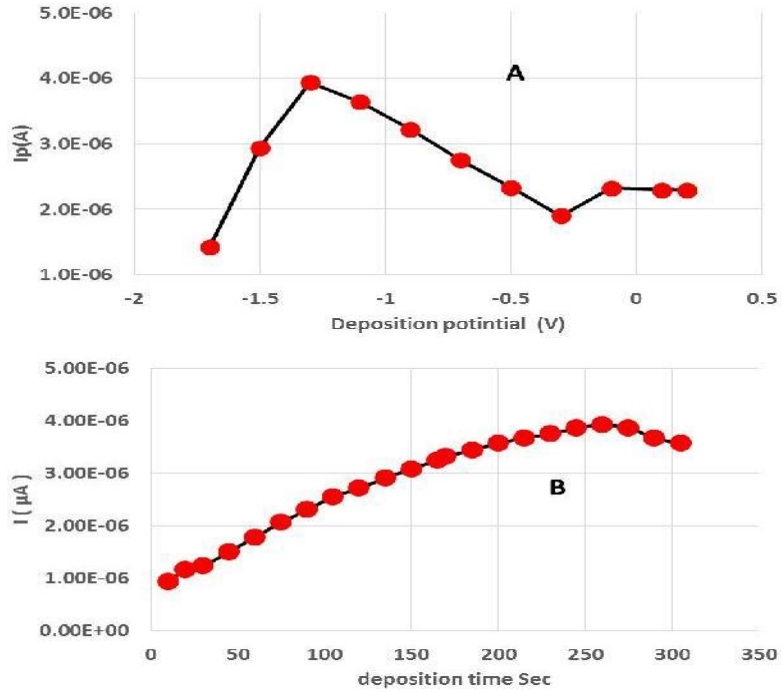

Fig. 7. Effect of accumulation potential (A) and accumulation time (B) on the peak current of 1.0×10^{-3} M TH in BR buffer of pH 10 at CPE.

Effect of accumulation potential and accumulation time (in case of CPE)

The effect of accumulation potential on peak current was studied over the range -1.7 to 0.2 V for 1.0×10-4 M TH in pH 10. It is obvious from Fig. 5(A) that the peak current reaches its maximum value at -1.3 V and the anodic current decreases as the value of accumulation potential increases. Therefore, the results obtained showed that the Ip attained shows the maximum value at accumulation potential -1.3 V. In addition, the influence of accumulation time on peak current studied. The anodic peak current increases as the accumulation time increases and reaches its maximum value at an accumulation time of 260 second and after this value, the anodic current decreases. Accumulation time 260 second is chosen as the optimum accumulation time as shown in Fig. 7 (B).

Mechanism suggested for oxidation of TH:

By applying Nernst equation using the formula ΔEp/ΔPH (slope) = 0.059/n, we can conclude that the number of electrons transferred is equal to 2 which is greater than number of protons transferred [26, 27] and the reaction is totally irreversible as discussed above. Upon which, we suggested the following mechanism (Fig. 8). We suggested that oxidation occurs on the nitrogen atom, which surrounded by two electron donating groups (2 methyl groups), thus increasing the basicity and facilitating the loss of electrons and the oxidation reaction.

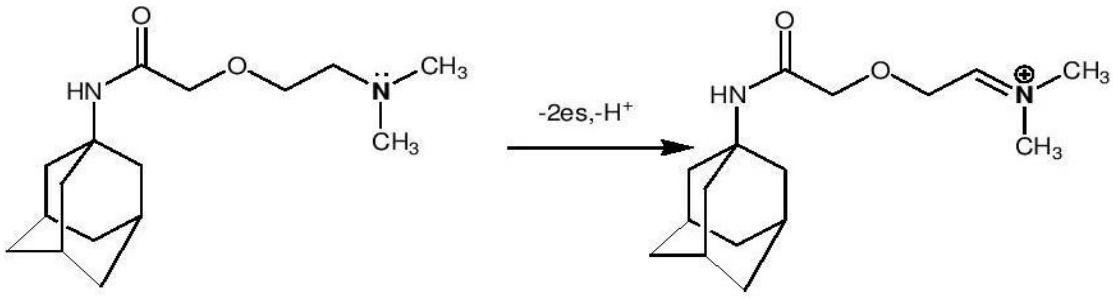

Fig. 8. The suggested mechanism for oxidation of TH.

Method Validation

The validity of the current method was evaluated and rated by examining and investigating the following specifications : accuracy, precision, linearity, range, LOD, LOQ, robustness, specificity and system suitability according to ICH guidelines [28, 29].

Linearity and range

Differential pulse and square wave voltammograms revealed that the peak current increased linearly with increasing concentrations as shown in Fig. 9 and 10. The results showed good linearity with regression parameters calculated according to Miller and Miller [30] as shown in table 1. Linearity relationship established over the concentration ranges indicated in Table 1 (Fig. 9). Statistical analysis of the data elucidated high values of correlation coefficient and small values of SD, SE and RSD, which proved the linearity of the proposed method over the specified concentration range (Table 1).

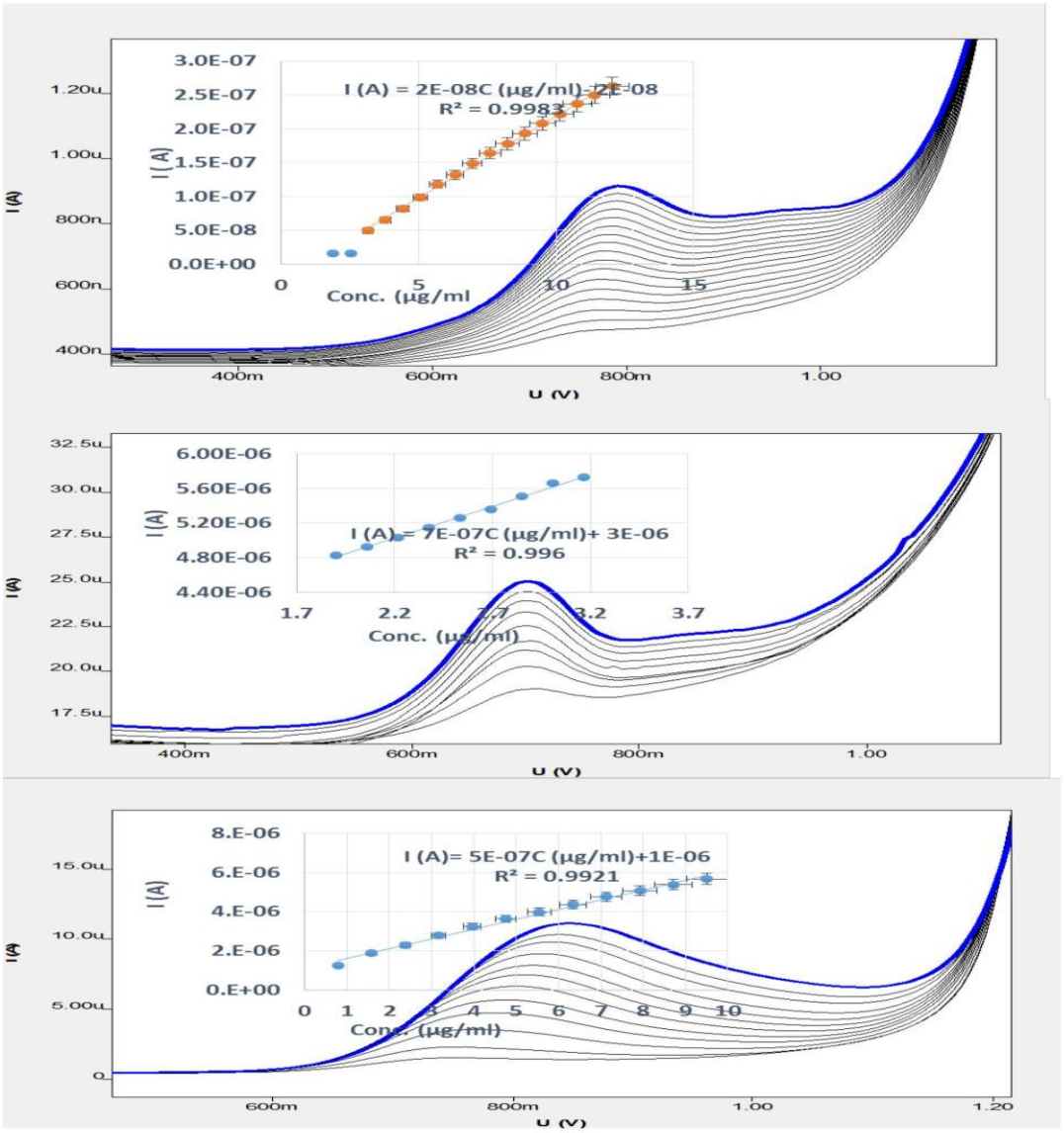

Fig. 9. DP voltammograms of TH at (a) GCE, (b) CPE and (c) PGE at υ = 50 mV/s, preconcentration time = 5 s, pulse amplitude (V) = 0.05, pulse time = 0.04 s, voltage step (V) = 0.006561 and voltage step time = 0.06561 s. Inset: The corresponding calibration plots.

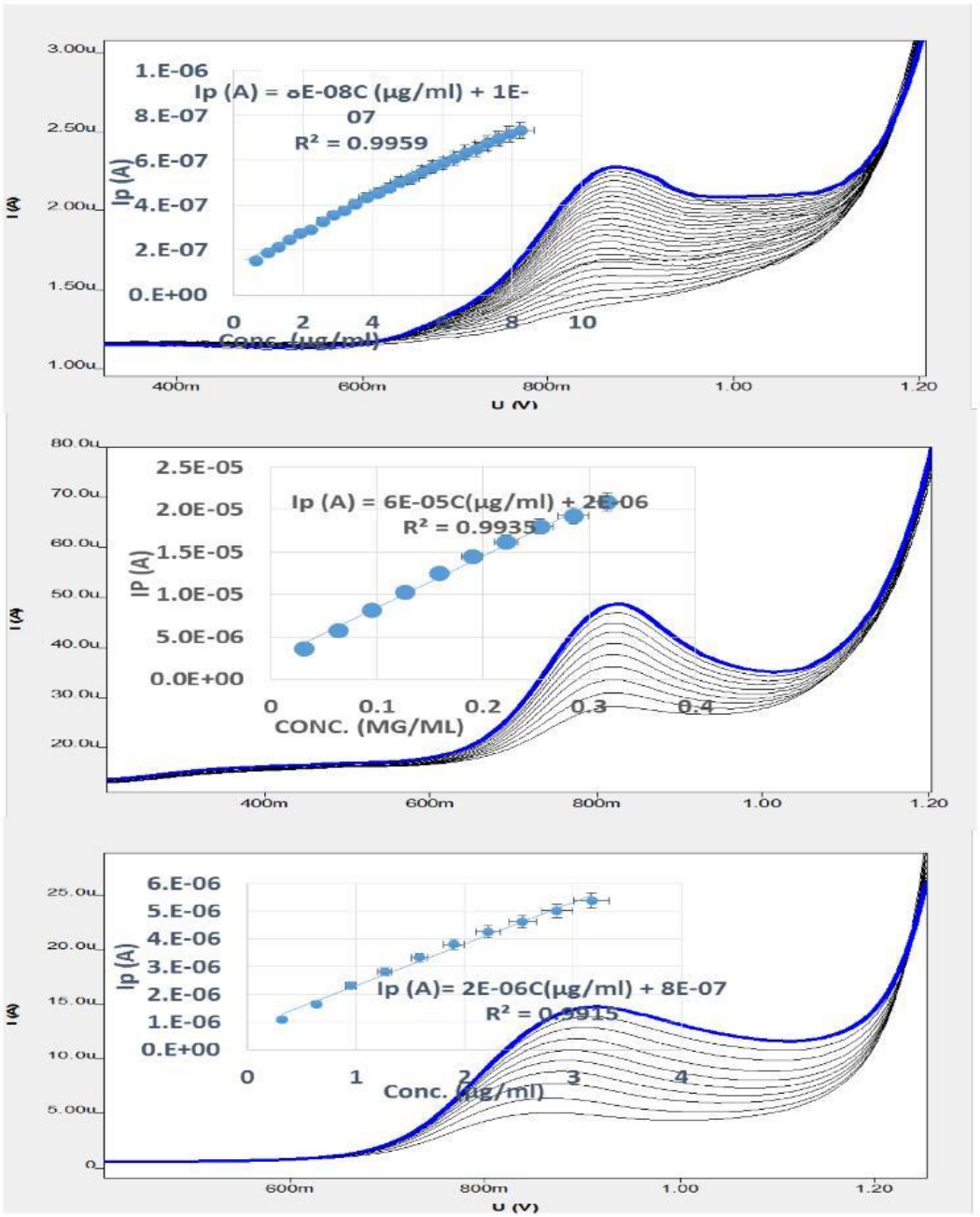

Fig. 10. SW voltammograms of TH at (a) GCE, (b) CPE and (c) PGE at υ = 50 mV/s, preconcentration time = 5 s, voltage step (V) = 0.006561, amplitude (V) = 0.019, sweep rate = 0.328 V/s. Inset: The corresponding calibration plots.

Limit of detection (LOD) and limit of quantification (LOQ)

LODs and LOQs were calculated and the results given in Table 1.

LODs and LOQs were calculated according to the following equations as stated by ICH guidelines [28, 30].

LOD = 3.3 σ / S

LOQ = 10 σ / S

Where σ = standard deviation of the response, S = slope of linearity.

Accuracy

Accuracy [31] is the measure of correctness of an analytical method. To prove the accuracy of the current method, the results of the assay of TH in pure form evaluated by the suggested Voltammetric method compared with those obtained using the reported non-aqueous titration method. Statistical comparison of the results obtained from both methods shows non-significant difference between the two methods.

Precision

Precision expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under normal conditions. The intra-and inter-day precision was evaluated by assaying freshly prepared solutions in quadruplicate on the same day and on four different days, respectively using the current method. The repeatability (intra-day) and reproducibility (inter-day) of the results obtained by means of the proposed DP voltammetric procedure were examined and the results indicated high accuracy and precision of the current method and could be adopted for quality control of TH (table 3).

Robustness

Robustness is the ability of a method to remain unaltered by small differences in method parameters. The robustness [32, 33] of a method is assessed by differing method parameters such as pH, ionic strength and temperature and determining the effect (if any) on the results of the method. The robustness of the proposed method shown by constancy of the peak current with deliberate small changes in the analytical parameters. The studied variables included; change in pH (±0.2), ambient temperature (±2 °c), and preconcentration time (5±5 s). The minor changes that may happen during normal experimental work did not affect the peak current intensity of the cited drug, thus proving that the method is robust.

Specificity and interference study (selectivity)

The specificity of the proposed method is demonstrated by its ability to determine TH in its pharmaceutical dosage form without any interference from other excipients present at the selected assay conditions. Thus, this method can applied by the quality control laboratories to quantify the drug and to check the formulation content uniformity.

Stability of stock and working solutions

Stock and working solutions of TH were stable for 3 weeks when kept in refrigerator at 4°C and stable for one week at room temperature. Zero changes in response appeared throughout the whole validation process.

Application to the pharmaceutical formula

The proposed method successfully applied for the determination of TH in its pharmaceutical dosage form. The results given in Table 2.

Table 1. Analytical parameters of the calibration curve of TH

Parameter	GCE		CPE		PGE	
	DPV	SWV	DPV	SWV	DPV	SWV
Anodic peak potential Ep (V)	0.780	0.823	0.755	0.803	0.798	0.841
Linearity range(µg/ml)	3.16-12.03	0.63-8.23	1.90-3.16	0.03-0.31	0.79-9.50	0.31-3.16
Slope	0.0242	0.0755	0.732	0.86	0.498	1.506
Intercept	-0.0234	0.131	3.417	2.332	1.122	0.803
(r^2)	0.998	0.995	0.996	0.993	0.992	0.991
%RSD*	0.789	0.328	1.764	0.584	1.184	0.601
SE	2.94	1.15	2.15	5.01	1.33	1.41
%Error	2.94	1.15	2.15	5.01	1.33	1.41
LOD (µg/ml)	0.36	0.45	0.09	0.02	0.80	0.28
LOQ (µg/ml)	1.21	1.52	0.29	0.08	2.67	0.94

* Five different concentrations of TH; number of replicates (n) = 5

Table 2. Determination of TH in gel

Parameter	Proposed method			Reference method
	Amount taken	Amount found	% found*	% found*
	(µg/ml)	(µg/ml)		
GCE	0.7	0.7	100	99.2
	0.9	0.9	100	99.5
	1.5	1.49	99.3	98.7
	3	2.97	99	98.5
Mean± SD			99.5±0.5	98.9±0.45
t-Test			0.06	
F-test			1.22	
CPE	0.05	0.05	100	
	0.15	0.15	100	
	0.25	0.25	100	
	0.3	0.298	99.3	
Mean± SD			99.8±0.35	
t-Test			0.01	
F-test			1.7	
PGE	0.6	0.6	100	
	1	0.99	99	
	2	1.98	99	
	3	2.96	98.6	
Mean± SD			99.1±0.59	
t-Test			0.3	
F-test			1.7	
t-Tabulated at p=0.05		2.44		
F-tabulated at p=0.05		9.27		

* Each result is the average of four separate determinations.

Table 3: Inter- and intra-days regression parameters for the Voltammetric determination of TH

Parameter	PGE			CPE			GCE		
Conc. µg/ml	3.5	4	4.5	2	2.2	2.4	1.5	2	2.5
Intra-day*	99.6±0.	99.5 ±0	99.9± 0.	101.3 ±0	99.8± 1.	99.1±0 .9	99.0±0 .7	100.3±2.	102.2± 1.0
	50	.93	30	.58	05	5	9	05	7
Inter-day*	99.3±0.	99.2 ±0	99.7± 0.	99.5± 0.	99.6± 1.	98.5±1 .2	98.5±0 .9	99.2±1.7	100.3± 1.5
	75	.8	62	95	57	8	5	9	9

*Each result is the average of four separate determinations.

CONCLUSION:

The suggested Voltammetric approach can be well applied for determination of TH in its pure form and pharmaceutical drug products using three different types of working electrodes; GCE, CPE and PGE [31], where the electro-oxidative behavior of TH was studied and showed one anodic irreversible peak. Different experimental conditions were examined and optimized; including pH, scan rate (sweep rate), accumulation time and accumulation potential. The proposed method enables a simple, sensitive, rapid and reproducible determination of TH compound without any intervention from any other ingredients present in the pharmaceutical formula. The present method is found to be practically precise, accurate, inexpensive and highly sensitive. Also the obtained results demonstrated that the proposed method could fortunately be selected and adopted for the quality control of TH.

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