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Electrochemical Behaviour of Trimebutine at Activated Glassy Carbon Electrode and its Direct Determination in Urine and Pharmaceutics by Square Wave and Differential Pulse Voltammetry

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Cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV) were used to explore the electrochemical behaviour of trimebutine (TB) at an activated glassy carbon electrode (GCE). Cyclic voltammetric studies showed a well defined oxidation wave at Britton-Robinson buffer (pH 5.0). The oxidation was reversible and exhibited diffusion controlled process depending on the pH. The mechanism of the oxidation process was discussed. A simple, precise, inexpensive and sensitive voltammetric method has been developed for the determination of the cited drug (TB). According to the linear relation between the peak current and the concentration, DPV and SWV methods were used for the quantitative determination of the cited drug in pharmaceutical dosage forms and urine samples. These two voltammetric techniques allow quantitation of TB over the concentration range from 1.0×10^{-6} to 1.1×10^{-5} and1.0×10^{-6} to 1.9×10^{-5} mol L^{-1}, respectively, using DPV and SWV methods. The correlation coefficients (r) were 0.998 and 0.999 for DPV and SWV methods, respectively. The linear response was obtained in Britton-Robinson buffer in the range of 1.0×10^{-6} - 2.0×10^{-5}mol L^{-1} for spiked urine samples. The limit of detection (LOD) and limit of quantification (LOQ) were 1.29×10^{-8} and $4.29\text{x}10^{-9}$ for DPV method and 2.34×10^{-9} and 7.79×10^{-9} for SWV method. The RSD for five measurements were 0.049 and 0.089 % using 200 mvs^{-1}scan rate. The method was applied successfully for the determination of TB in dilute urine samples and dosage forms and it compared well with the reported HPLC method. It showed good recovery and reproducible results.

Keywords: Cyclic voltammetry- Differential pulse voltammetry- Trimebutine-Urine- Tablets.

1. INTRODUCTION

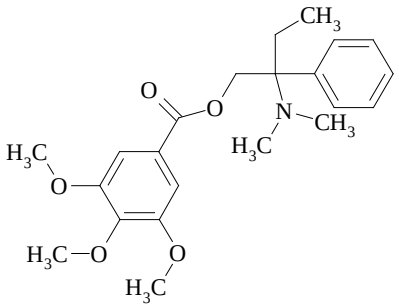


Figure 1. Structure of 3,4,5-trimethoxybenzoic acid 2(dimethylamino)-2-phenyl butylester.

Trimebutine (TB) 3,4,5-trimethoxybenzoic acid 2(dimethylamino)-2-phenyl butylester (Figure 1) is a drug with antimuscarinic and weak muopioidagonist effects. It is an effective antidiarrheal drug which is used as adjuncts in the symptomatic treatment of diarrhea.The major product from drug metabolism of trimebutine in human beings is nor-trimebutine, which comes from removal of one of the methyl groups attached to nitrogen. Both Trimebutine and its metabolite are commercially available [1]. Several techniques have been used to determine TB including voltammetry [2], spectrophotometry [3], mass spectrometry [4], liquid chromatography [5,6], high performance liquid chromatography [7,8] and capillary electrophoresis [9-10]. The reported methods were influenced by interference of endogenous substance and potential loss of drugs and requiring a sophisticated and expensive instrumentation. Development of a new method capable of determining drug amount in pharmaceutical and biological dosage forms is important. Electroanalytical techniques have been used for the determination of a wide range of drug compound with the advantages that there is no need for derivatization and that these techniques are less sensitive to matrix effects than other analytical techniques. Additionally, application of electrochemistry includes the determination of electrode mechanism. Redox properties of drugs can give insights into their metabolic fates or their in vivo redox processes or pharmacological activity [11-17]. The goal of this work was the development of new voltammetric method for direct determination of TB in pharmaceutical dosage forms, raw materials and spiked human urine samples without any time-consuming extraction or evaporation steps prior to drug assay. This paper describes fully validated, simple rapid, selective and sensitive procedures for the determination of TB employing DPV, SWV and CV. This work was also aimed to study the voltammetric behaviour and oxidation mechanism of the cited drug using this technique.

2. EXPERIMENTAL

2.1 Reagents and apparatus

TB as a pure material was supplied from Amoun Company Cairo, Egypt. Dosage forms of TB were purchased from Global Napi Pharmaceutical Co. **A** stocksolution of 1.0×10^{-3} mol L^{-1} TB was freshly prepared by dissolving the weighed amount in acetonitrile, and stored in refrigerator in PVC

container. 0.2 mol L^{-1} phosphate buffer between pH 2-12,0.04 molL^{-1} Britton- Robinson buffer between pH 2-11 and 0.2 mol L^{-1} acetate buffer between pH 3.5-5.7 were used as the supporting electrolytes. Other chemicals, all of analytical- reagent grade (Merck) were used. Standard solutions were prepared by serial dilution of the stock solution with selected supporting electrolyte. The calibration curves for DPV and SWV analysis were constructed by plotting the peak current against TB concentration. The ruggedness and precision were checked at different days, within day and between days. Relative standard deviations were calculated to check the ruggedness and precision of the method. Voltammetric measurements were obtained using the electrochemical analyzer Computrace system with 797VA Computrace software (1.0) from Metrohm, Switzerland. A three-electrode cell was employed incorporating a glassy carbon stationary electrode as a working electrode (Mini glassy carbon disk electrode of the active zone: 2.8 mm for ELCD641/656), Ag/AgCl (3 mol L^{-1}KCl) reference electrode and a platinum wire counter electrode. The data were treated with Microsoft Excel windows 7.0. A Mettler balance (Toledo-AB104) was used for weighing the solid materials. The pH measurements were performed using digital Jenway 3330 Research pH meter, glass electrode system was calibrated before and after each series of measurements under the same condition. A micropipette (Eppendorf-multipette plus) was used throughout the present experimental work. Deionized water used throughout the present study was supplied from a burette still plus deionized connected to a Hamilton-Aqua-Metric deionized water system. All measurements were carried out at ambient temperature of the laboratory.

For analytical application, the following parameters being employed: DPV-pulse amplitude 50 mV, pulse width 50 ms, scan rate 200 mVs^{-1}; SWV-pulse amplitude 25 mV, frequency 15 Hz, potential step 4 mV, electrochemical analyzer does the background subtraction automatically. For CV, the initial and final potential were variable, depending on the pH value and the cut-off the electrolyte. Scan rate measurements in the range of 5-250 mVs^{-1} were carried out.

2.2. Pre-treatment of glassy carbon electrode

The electrode was pretreated by cycling a square-wave potential with a frequency of 350 Hz between the potential limits of ±6 V followed by the application of triangular potential sweep between ±6 V (frequency 3500 Hz) in 0.1 mol L^{-1} potassium nitrate solution. Finally, the electrode was subjected to an electrochemical pretreatment by applying a potential of +1.5 V for 5 min and then -1.0 V for 2 s in 0.1 mol L^{-1} potassium nitrate solution. These steps were repeated until the voltammetric response of the electrode became reproducible. At the end of the procedure, the electrode surface was so stable that for 40 measurements, the electrochemical pretreatment alone was sufficient before each scan [18, 19].

2.3. Pharmaceutical dosage form assay procedure

Ten tablets of triton (each tablet contains 100 mg TB) were accurately weighed and finely powdered by pestle in a mortar. A weighed portion of this powder equivalent to 1.0 $x10^{-3}$ mol L^{-1} of

TB was transferred into a 100 mL calibrated flask and completed to the volume with acetonitrile. The content of the flask was sonicated for 10 min to achieve complete dissolution. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with the selected supporting electrolyte in order to obtain a final solution. The amount of TB per tablet was calculated using linear regression equation obtained from the calibration curve of pure TB.

2.4. Analysis of spiked urine samples

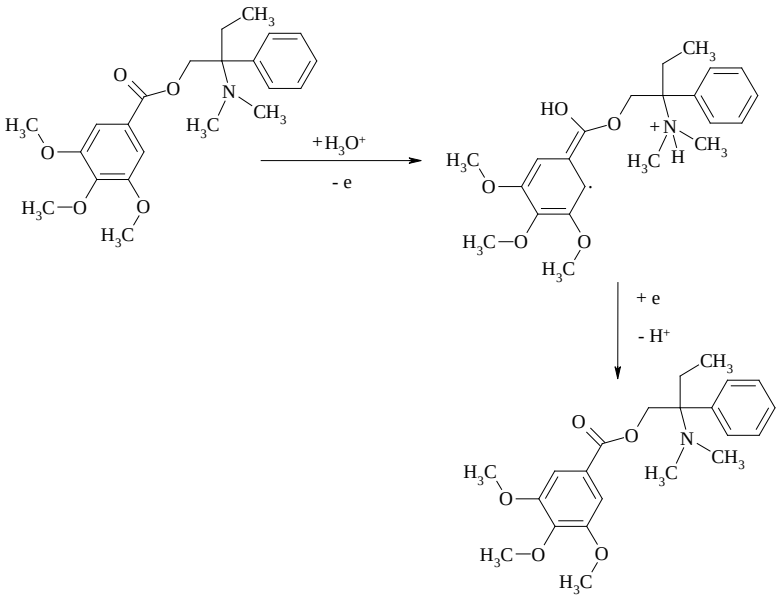
The urine samples were taken from healthy individuals immediately before the experiments. An aliquot volume of urine sample was fortified with TB dissolved in 0.5 mL acetonitrile as endogenous substance precipitating agent, to achieve final concentration of $1x10^{-3}$ mol L^{-1}. Then the volume was completed to 2 mL with the same urine sample. After overtaxing for 10 min, and centrifuged for 5 min at 5000 rpm for getting rid of protein residues, the supernatant was taken carefully. Appropriate volumes of this supernatant were transferred into the volumetric flask and diluted up to the volume with Britton- Robinson buffer of pH 5.0. The concentration of TB was varied in the range of 1.0×10^{-6} - 2.0×10^{-5} mol L^{-1}in human urine samples. The amount of TB in spiked human urine samples for the recovery studies was calculated from the related calibration equation.

2.5. Recovery studies

To study the accuracy and reproducibility of the proposed techniques, recovery experiments were carried out using the standard addition method. In order to know whether the excipients show any interference with the analysis, known amounts of pure TB were added to pre-analyzed tablet formulation and the mixture was analyzed by the proposed method. After five repeated experiments, the recovery results were calculated using the calibration equation.

3. RESULTS AND DISCUSSION

Anodic cyclic voltammogram for the oxidation of TB in Britton-Robinson buffer at activated glassy carbon electrode is shown in Fig. (2). It is clear that the voltammogram exhibits one anodic and cathodic peaks at 288 and 342 mV, respectively, which shows a quasi- reversible behaviour, with peak- to - peak separation ($\Delta Ep = | Epc-Epa|$) of 54 mV. The voltammogram is characterized by reversible redox couples governed by $\Delta Ep = (59\ m/n)$; where m is the number of protons and n number of electrons involved in the reaction [20]. Thus, the results suggest the redox couple which shows reversible behaviour with equal number of protons and electrons participated in the working buffer. The TB may undergoes protonation with bond cleavage and one electrone loss in the first oxidation step. Then the oxidized product is reduced via gaining electrone nad deprotonation of the oxidized product. The proposed mechanism of the oxidation reduction reaction can be given as shown below:



Scheme 1. Proposed mechanism of the oxidation reduction reaction of TB drug.

The effect of scan rates on the peak current at GCE in Britton-Robinson buffer of pH 5 was investigated by cyclic voltammetry (Fig. 3). Scan rate studies were carried out to assess whether the process at the glassy carbon electrode was under diffusion or adsorption controlled process as shown in Fig. (4).

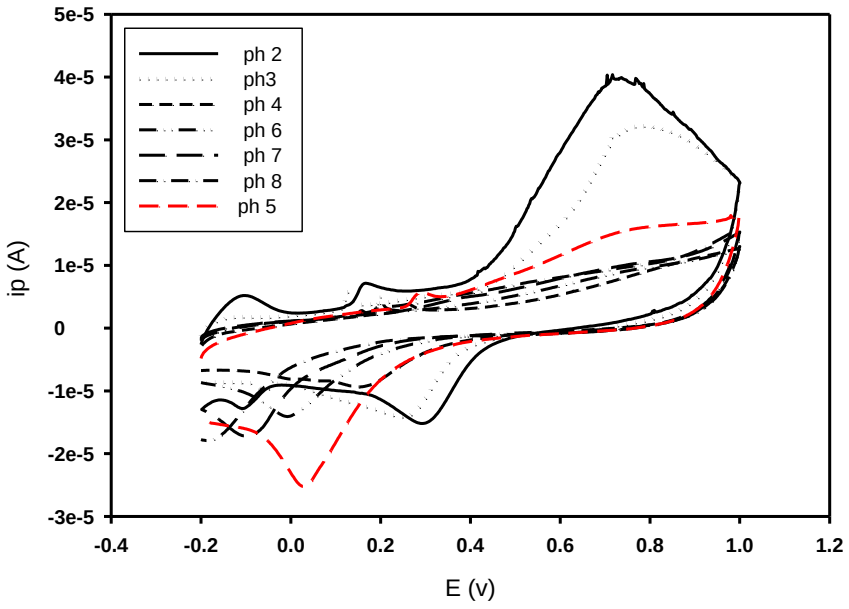


Figure 2. Cyclic voltammograms at different pH.

The anodic and cathodic peak current of TB increases linearly with the square root of scan rate, in the potential range from 200 to 1000 mV, with Regression r = 0.998. A plot of logarithm of peak

current versus logarithm of scan rate gave a straight line with a slope very close to the theoretical value of 0.5, which is expressed for an ideal reaction of diffusion controlled electrode process (Fig. 5) [21]. Also the anodic and cathodic peak potentials of the electrode were shifted toward positive and negative potential, respectively. This may be attributed to the accumulation of the oxidation or reduction products on the electrode surface.

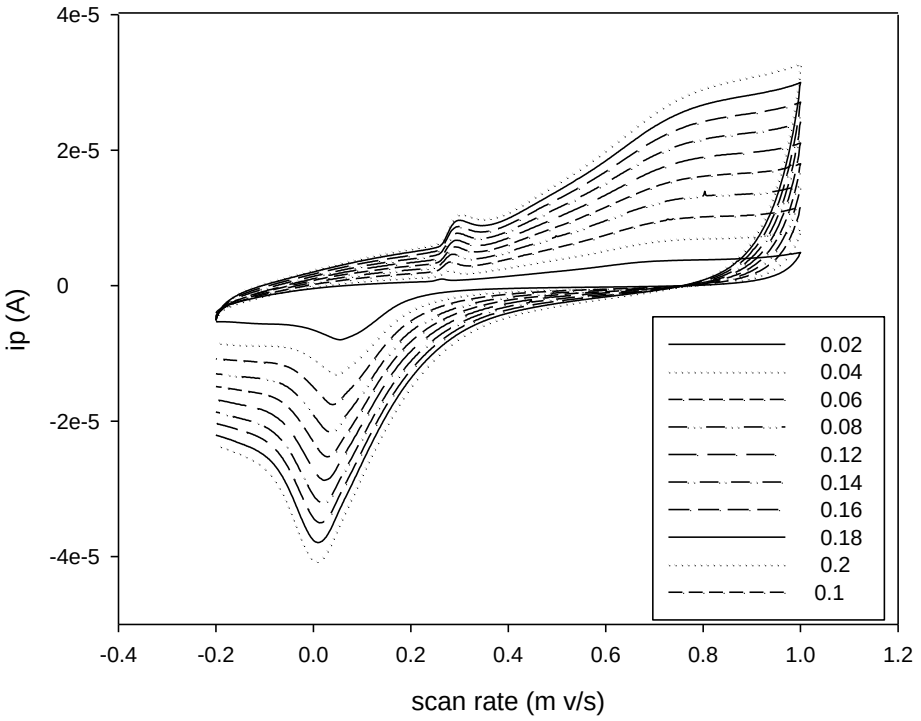
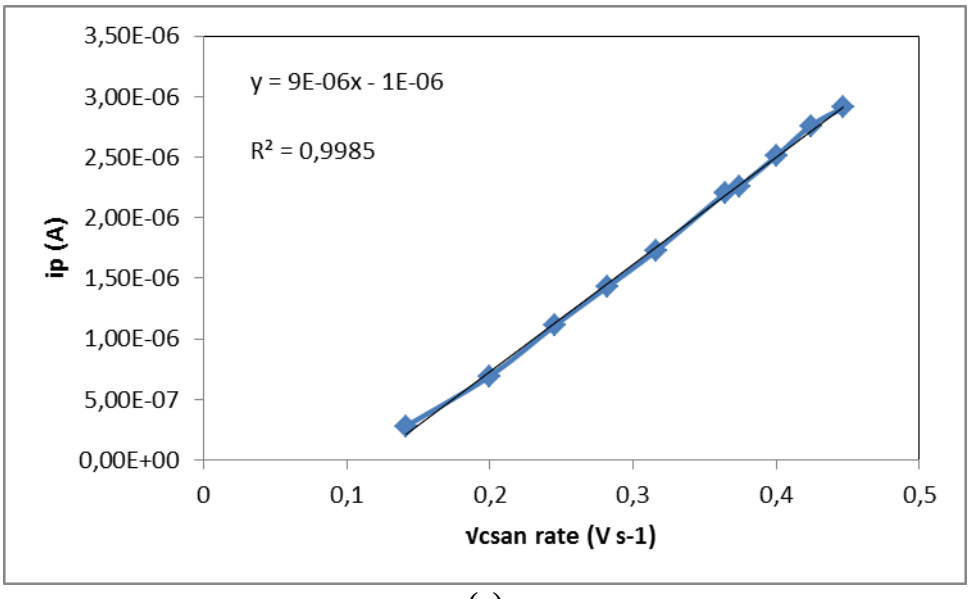
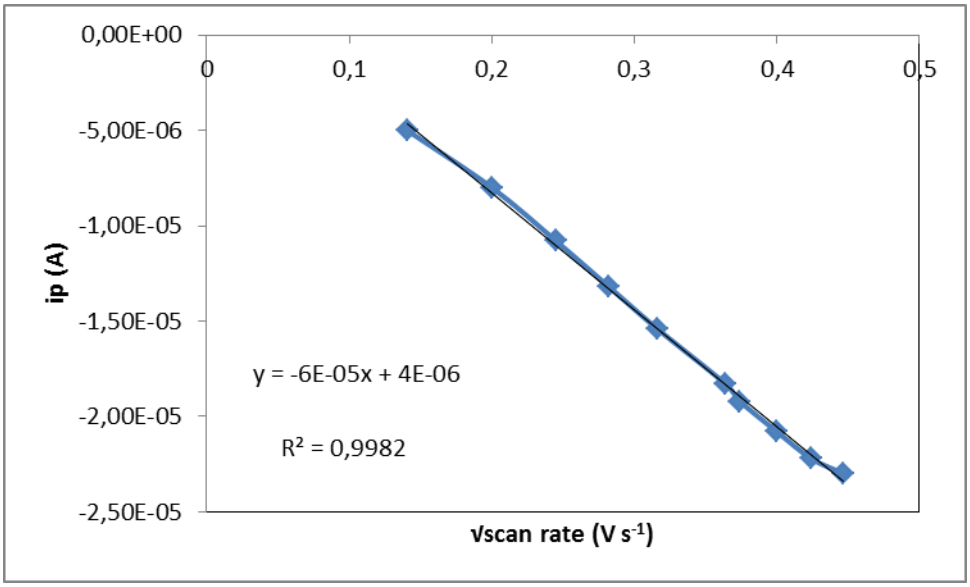


Figure 3. The effect of scan rates on the peak current at GCE.



(a)



(b)

Figure 4. The anodic (a) and cathodic (b) peak current of TB with the square root of scan rate.

Therefore, the anodic and cathodic peak separation increases. In this study, 200 mV s^{-1} was chosen for scan rate because at this value the sensitivity was relatively high and voltammetric curves were well shaped with a relatively narrow peak width.

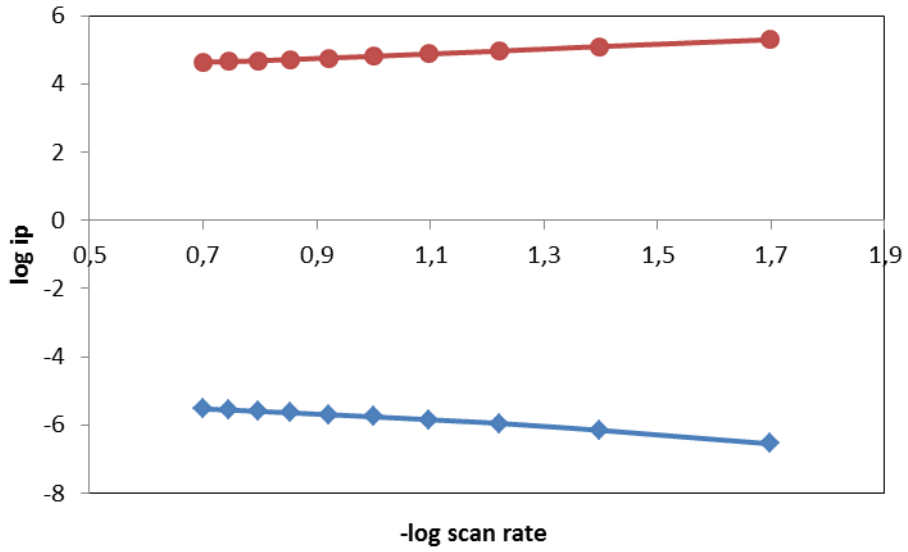


Figure 5. Relation between log ip (μA) and log scan rate (vs^{-1}) for oxidation of TB drug at activated glassy carbon electrode.

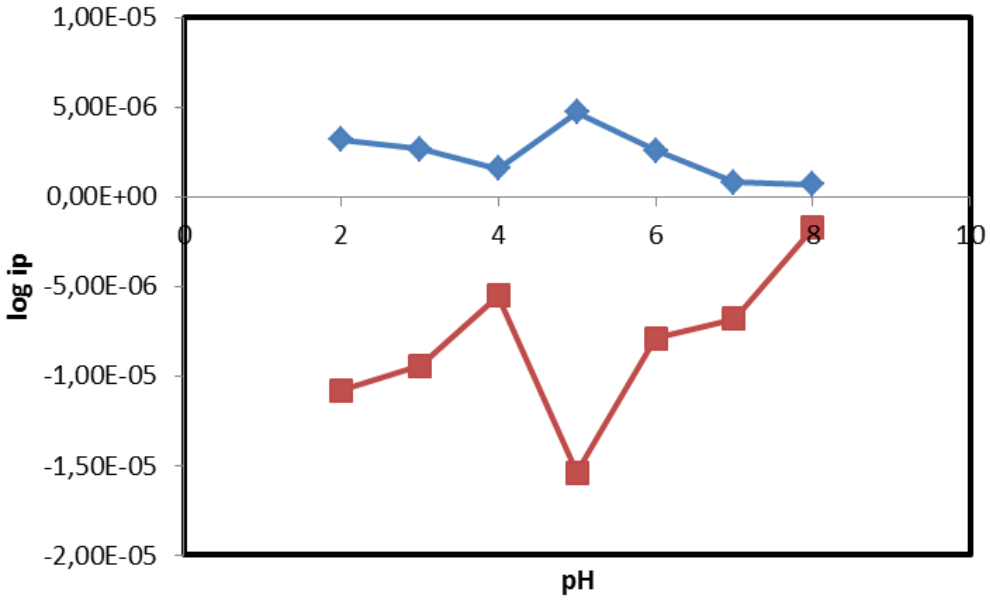


Figure 6. The effect of pH on the current peak.

The influence of pH and type of supporting electrolyte on the peak current was examined using cyclic voltammetry. The composition of the supporting electrolyte was evaluated at 1.0×10^{-3} mol L^{-1} TB solution in various electrolytes, such as acetate, phosphate and Briton-Robinson buffers of different ionic strength in the range of 0.04- 0.2 mol L^{-1}. It was found that, the maximum peak size was obtained at pH 5 in Britton-Robinson buffer which allows wider range of determination than acetate [2] and phosphate buffers. Hence it was used as a favourable buffer throughout the study. Fig (6) shows the effect of pH on the current peak in a Briton-Robinson buffer of pH range from 2 to 8 for 2.0×10^{-6} mol L^{-1} TB.

The effect of solution pH on peak potentials of TB at GCE was also investigated. Cyclic voltammograms at different pH values of 2-8 were shown in Fig. 2. These data show that an increase in pH of the solution caused shift in the oxidative peak potential to the negative direction, indicating that the electrode process is influenced by protonation reactions. A linear correlation between the peak potential and solution pH was obtained as shown in Fig. (7) with a linear equation and correlation coefficient of:

Epa (mVs^{-1}) = 0.468-0.078 pH r = 0.991

The slope was found to be 0.078 mV/pH which suggested that the number of proton taking part in the electrode reaction is similar to the number of electrons. Hence, the oxidation of TB involves one electron and one proton [20,21].

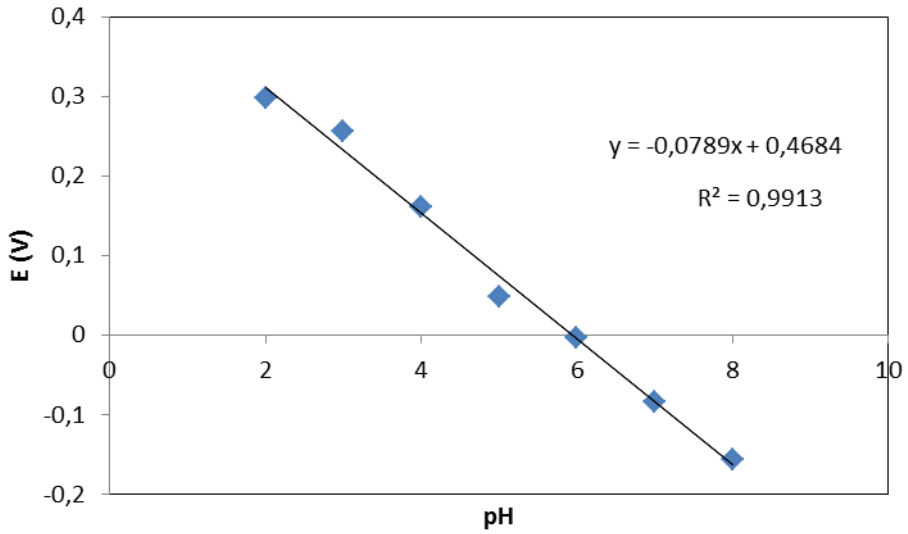


Figure 7. A linear correlation between the peak potential and pH.

3.1. Optimization of technique parameter

Various electrolytes were examined as discussed above. The best results with respect to signal enhancement and sharp peak accompanied by sharper response were obtained with Britton-Robinson buffer of pH 5.0. This supporting electrolyte was chosen for the subsequent experiments. Since DPV and SWV have much higher current sensitivity and better resolution than cyclic voltammetry [22]. They have been selected in order to develop voltammetric procedures for drug determination, since the peaks were sharper and better defined at lower concentration of TB than those obtained by cyclic and linear sweep voltammetry with a lower background current, resulting in improved resolution. DPV and SWV are effective and rapid electroanalytical techniques with well- established advantages, including good discrimination against background current and low detection limits [16-17]. Two calibration graphs from the standard solution of TB according to the procedure described above were constructed by using DPV and SWV techniques. A linear relation (Figs. 8, 9) in the concentration range between 1.0×10^{-6} to 1.1×10^{-5} and 1.0×10^{-6} to 1.9×10^{-5} mol L^{-1} by using DPV and SWV methods, respectively, was found, indicating that the response was diffusion controlled in this range. Above this concentration a loss of linearity was probably due to the adsorption of TB on the electrode surface.

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated on the peak current using the following equations [23, 24]:

LOD= 3s/m LOQ= 10s/m

Where s is the standard deviation of the peak currents (three runs) and m is the slope of the calibration curve (Table 1).

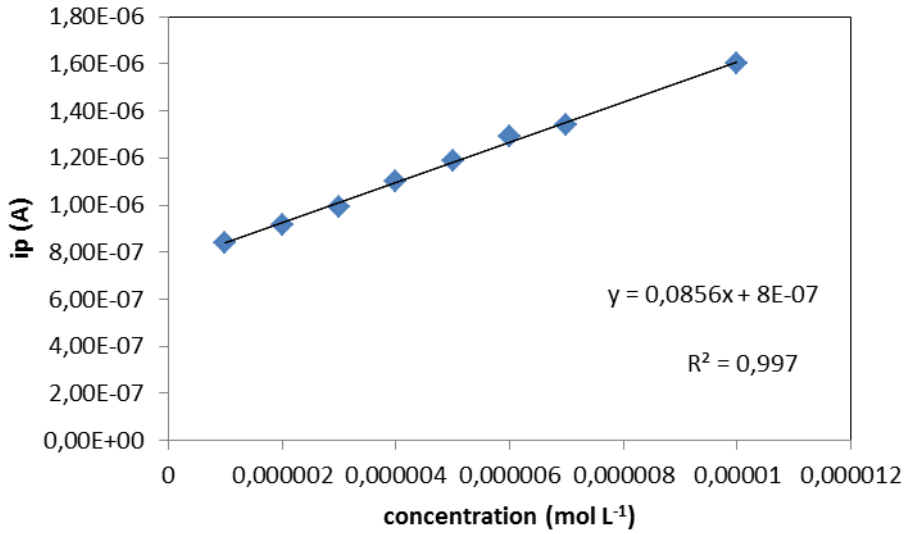


Figure 8. Calibration graphs of TB drug by using DPV method.

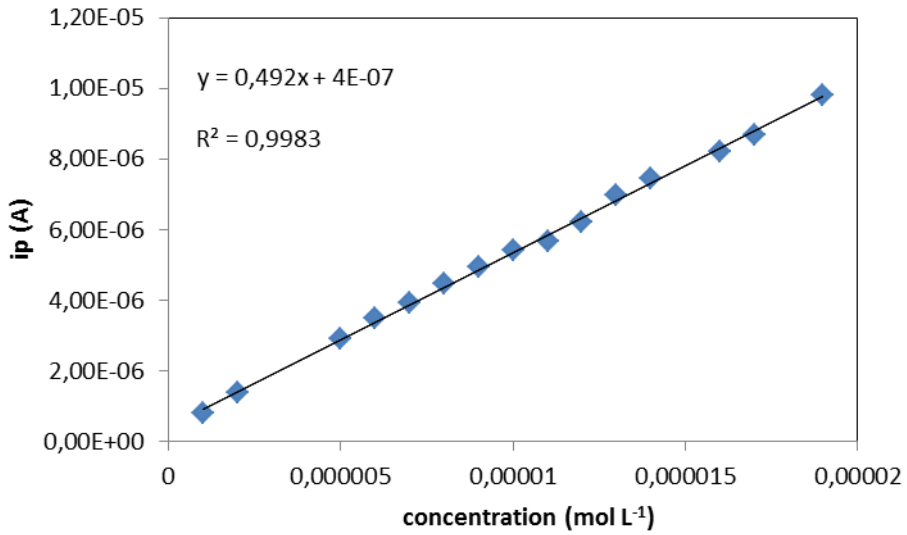


Figure 9. Calibration graphs of TB drug by using SWV method.

Repeating four experiments on 5 x10^{-5} mol L^{-1} TB for both techniques was tested for evaluating the repeatability and reproducibility of peak potential and peak currents. Repetition of sample analysis after 72 h period did not show any significant change in the results.

The selectivity of the optimized procedure was examined in the presence of some common excipients to monitor the interference effect. A mean recovery of 1.2 x 10^{-8} mol$L^{-1}$of the cited drugs ranging from 98.7 % to 101.2% was obtained. The proposed method can be considered to be selective. The robustness of the results of the procedure is the ability to remain unaffected by small change in its operational parameters such as pH (Table 2). In the present work this was examined by studying the effect of a variation of pH. The recovery values were not significantly affected by this variation and consequently the optimized procedure was reliable for the assay of drugs. It could be robust. The

ruggedness is the degree of reproducibility of the results obtained by analysis of the same sample under a variety of normal test conditions, such as different laboratories, analysts and lots of reagents.

Table 1. Analytical parameters of TB drug by using DPV and SWV methods.

Parameters	Differential pulse voltammetry (DPV)	Square wave voltammetry (SWV)
concentration (mol L^{-1})	1.0×10^{-6} to 1.1×10^{-5}	1.0×10^{-6} to 1.9×10^{-5}
SD RSD %	3.67×10^{-10} 0.0489	3.84×10^{-10} 0.0885
Slope of regression line (a)	0.0856	0.492
Intercept of regression line (b)	7.5×10^{-7}	4.35×10^{-7}
Correlation coefficient (r)	0.998	0.999
SEE	1.47×10^{-8}	1.11×10^{-7}
LOD (mol L^{-1})	1.29×10^{-8}	2.34×10^{-9}
LOQ (mol L^{-1})	4.24×10^{-8}	7.73×10^{-8}

Table 2. The robustness and the ruggedness of the condition of the proposed method for the determination of investigated drug at activated glassy carbon electrode.

Variable	Drug	Recovery % ± RSD
Robustness Result at pH = 5	triton	99.11 ± 0.59
Ruggedness Analyst-1	triton	98.51 ± 0.51
Ruggedness Analyst-2	triton	99.00 ± 0.63

This was examined by applying the proposed procedures to an assay under experimental conditions using different analysis. The results obtained due to lab. (1) to lab. (2) and even day to day were found to be reproducible, since there is no significant difference between the recovery and SD values[19].

3.2. Determination of TB in tablet

On the basis of above results, both DPV and SWV methods were applied to the direct determination of TB in tablet dosage forms, using the related calibration straight lines after an adequate dilutions. The results show that the proposed methods were successfully applied for the assay of TB in its pharmaceutical dosage forms (Table 3).

Table 3. Statistical parameters of pharmaceutical dosage form assay of the investigated drug by the proposed SWV method and official method.

Sample	Drug	[Drug] taken µg mL^{-1}	Proposed method ± RSD%, n=5	Official method ± RSD%, n=5	F-test	t-test
trimebutine	Triton (100 mg/tablet)	30	100.3 ± 2.13	99.41± 2.00	1.57	2.25
		70	99.98 ± 1.11	100.2 ± 1.32	1.78	2.45
		90	99.98 ± 0.94	99.74 ± 1.00	1.85	2.23

Tabulated t-value =2.77, Tabulated F-value = 6.39
at 95% confidence limit, n = 5, degree of freedom = 4.

The accuracy of the method was determined by its recovery during spiked experiments. Recovery studies were carried out after the addition of known amounts of the pure drug to various pre-analyzed formulation of TB. According to the results, excipients presented in tablet do not interfere with the analysis (Table 3). To prove the absence of interference by excipients, recovery studies were carried out. The results demonstrated the validity of the proposed method for the determination of TB in tablet. These results reveal that both methods had adequate precision and accuracy and consequently can be applied to the determination of the cited drug in pharmaceuticals without any interference from the excipients.

3.3. Determination of TB in spiked urine samples

Acetonitrile and methanol were tried as a urine precipitating agents. Also, different amounts of acetonitrile were tried. The best results were obtained using 0.5 mL acetonitrile. The measurement of TB in urine sample was performed as discussed in the previous section. The applicability of the proposed methods to human urine sample, the calibration graph was obtained (Fig. 10). Analysis of drugs from biological samples usually requires extensive time-consuming sample preparation, use of expensive organic solvents and in some times, use of other chemicals. In this study, the urine samples were precipitated by the addition of acetonitrile, which is centrifuged at 5000 rpm and the supernatant was taken and diluted with the supporting electrolyte and directly analyzed. It has been found that, using both proposed techniques, no sample pre-treatment was required, other than precipitation and dilution steps. No oxidation compounds and no extra noise peaks present in biological material peak

occurred in the potential range where the analytical peak appeared. Stability of urine samples kept in refrigerator (+4 ºC) was tasted by making five consecutive analyses of the samples over a period of approximately 5h. There were no significant changes in the peak currents and potentials between the first and last measurements.

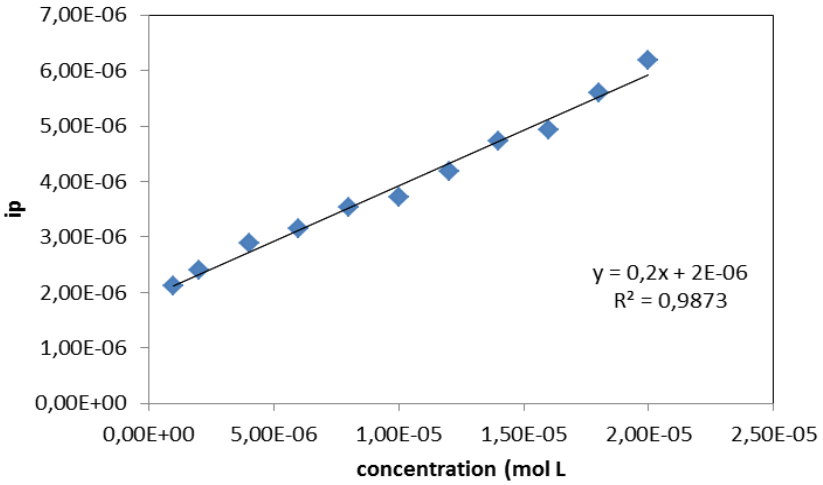


Figure 10. Calibration graphs of TB in spiked urine samples by using SWV method.

In conclusion, the electrochemical behaviour of TB on glassy carbon electrode was established. The voltammetric anodic peak at pH 5.0 most probably is due to diffusion controlled process. The electrochemical process is reversible and pH dependent. A validated differential pulse and square wave voltammetric techniques have been developed and successfully applied to the estimation of TB in pharmaceutical formulations and urine samples. DPV and SWV are effective and rapid electrochemical techniques with well- established advantages, including good discrimination against background current and low detection limits [17]. The proposed method is direct and more sensitive than other reported methods [25]. Furthermore, the proposed method is simple, accurate precise and does not need the elaborated treatment and tedious extractions required in chromatographic methods. The main advantages of the method are rapidity as it requires less than 5 min to run sample, no pretreatment low cost, sensitivity (very low LOD and LOQ), simplicity and non- destructive nature. The common interfering substances in the real sample, like ascorbic acid or urate, yield the anodic peaks at much less positive potential than TB. For this reason in this study, these kinds of interfering substances do not interfere.

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