Anal. Bioanal. Electrochem., Vol. 4, No. 2, 2012, 197 - 211

Analytical & Bioanalytical Electrochemistry

Full Paper

Electrochemical Behavior and Determination of Cilostazol in Pure, Urine and in Pharmaceutical Formulations

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Received: 6 March 2012 / Accepted after minor revision: 14 April 2012 / Published online: 30 April 2012

Abstract- The adsorptive and electrochemical oxidation behavior of Cilostazol has been studied at CPE and GCE in Britton - Robinson (BR) (pH 2.0 to 12.0). The anodic oxidation at 663 and 682 mV for CPE and GCE respectively. Differential pulse voltammetry was used to determine Cilostazol in the pure and in pharmaceutical formulations. The linear calibration was obtained from: 4.0×10^{-7}–6.40×10^{-6} M and 5×10^{-7}–8.7×10^{-6} M for carbon past and glassy carbon electrode. The percentage recoveries were found in the ranges: 99.50-100.80%, 99.20 -100.10% at carbon past and glassy carbon electrodes, respectively. The RSD for five measurements were found in the ranges: 0.403-0.742% and 0.657-0.84 %,. The method was applied to determine Cilostazol has in dilute urine sample and dosage forms and compared with official methods.

Keywords- Carbon Past Electrode, Glassy Carbon Electrode, Differential Pulse Cyclic Voltammetry, Cilostazol Assay, Dosage Forms

1. INTRODUCTION

Cilostazol is 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy]-3,4-dihydro-2(1H)quinolinone. The empirical formula of Cilostazol is $C_{20}H_{27}N_5O_2$ and its chemical structure is shown in

Fig.1. Cilostazol is a quinolinone derivative and medication has been used in treatment claudication in individuals with peripheral vascular disease. It is manufactured by Otsuka Pharmaceutical Co.Ltd., Japan, [1].There are several methods for determination of Cilostazol in pure and pharmaceutical preparations [2–11]. However, some of these methods need expensive equipment and/or are time consuming. There analytical methods were not involving electro behavior of Cilostazol. Therefore, an electro determination method was developed and validated for the simultaneous analysis of Cilostazol. This work aimed to develop alternative and a new electro determination method. Electrochemical methods have proved to be fast, accurate, precise, simple, and sensitive for the determination of organic molecules that undergo oxidation or reduction reactions, including drugs and related molecules in pharmaceutical dosage forms and biological fluids [12-18]. The aim of this study is to establish and optimize the experimental conditions for the determination of Cilostazol in pure form, pharmaceutical forms and urine by using cyclic voltammetry and differential pulse voltammetry (DPV) techniques.

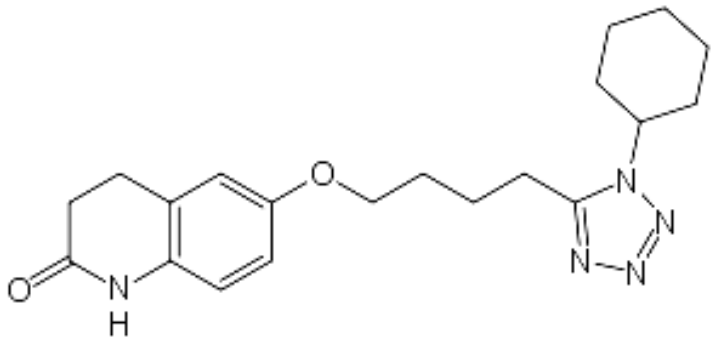

Fig. 1. Structure of Cilostazol

2. EXPERIMENTAL

2.1. Apparatus

All voltammetric experiments were carried out using a Metrohm computrace voltammetric analyzer model 797 VA with Software Version 1.0 (Metrohm Switzerland) is the name of the control software for the PC-controlled 797 VA Computrace System for voltammetric analysis. With a three-electrode configuration: a carbon paste electrode or glassy carbon disc electrode as working electrode (mini glassy carbon disk electrode of the active zone: 2.8 mm, for ELCD 641/656), a Ag/AgCl (3 ml^{-1} KCl) as reference electrode and a platinum wire counter electrode were used. A digital pH/mV meter (JEANWAY 3510) with a glass combination electrode was used for the preparation of the buffer solution.

A micropipette (Eppendorf-multipette plus) was used throughout the present experimental work.

2.2. Reagents

Cilostazol was supplied from Otsuka maser Pharmaceutical, Egypt. Stock solutions of 10^{-3} M was prepared by dissolving an appropriate weighed of Cilostazol in 25 ml methanol then complete the appropriate volume (100 ml) with bidistilled water . The stock solution was stored in a refrigerator. Britton-Robinson (BR) buffer solutions (2.0-12) [19] were used as supporting electrolyte. All solutions were prepared by using analytical grade reagents in bidistilled water.

2.3. Working electrodes

To improve the sensitivity and resolution of the voltammetric peaks, the glassy carbon electrode (GCE) was polished manually with 0.5 μm alumina powder on a smooth polishing cloth prior to each electrochemical measurement. Then, it was thoroughly rinsed with methanol and double distilled water, and gently dried with a tissue paper.

2.4. Preparation of the carbon paste working electrodes

The carbon paste was prepared by mixing of 0.5 g graphite powder with 0.3 ml of paraffin oil in a mortar with a pestle. a portion of composite carbon paste was packed into the hole of the insulin syringe body with diameter 3.0 mm which contain copper wire contacted the apparatus and The tip of the electrode was polished with a weighing paper until it had a shiny appearance .

2.5. Preparation of tablet sample assay

Ten tablets of Cilostazol were crushed into a fine powdered in a mortar. A suitable amount of this powder was accurately weighed and then dissolved in 25 ml methanol. It was sonicated for 5 minutes. The content was allowed to settle after stirring magnetically for 5.0 min. The sample solution was filtered through a whatman no.42 filter paper. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquid and diluting them with bidistilled water in order to obtain a final solution of 10^{-3} M Cilostazol. Each solution was transferred to a voltammetric cell and the voltammograms were subsequently recorded following the optimized conditions. The content of the drug in tablet was achieved by the standard addition method.

2.6. The optimizations

To obtain the optimum pH, an appropriate amount of Cilostazol working standard solution 10^{-3} M was placed in the electrolytic cell, which contained 25 ml of BR buffer solution and the cyclic voltammogram was recorded. The experiment was repeated by using buffer solutions of different pH values (2.0-12) and the optimum pH was obtained.

We study the effect of different types buffer such as acetate, borate and BR buffer on the peak current (I_p) of Cilostazol, the working electrode was immersed in buffer solution of the optimum pH containing an appropriate amount of the drug stock solution (10^{-3} M), the cyclic voltammogram was recorded .

The study the effect of scan rate (υ) on the peak current (I_p) of Cilostazol, the working electrode was immersed in the optimum buffer solution containing an appropriate amount of Cilostazol standard solution 2.5×10^{-5} M, and the cyclic voltammograms were recorded at different scan rates over the scan range 10-250 mV/s. Plot log I_p *vs*. log υ to know the nature of the process, diffusion controlled process or adsorption controlled process .

To study the effect of accumulation time, the working electrode was immersed in the optimum buffer solution containing an appropriate amount of Cilostazol standard solution 2.5×10^{-5} M to select times with stirring at 1200 rpm at open circuit condition. After accumulation, the cyclic voltammograms were recorded then plot the peak current (I_p) *vs*. time to obtain the optimum accumulation time.

The optimum instrumental conditions for the determination of Cilostazol by using DPV method were chosen from a study of the variation of the peak current with pulse amplitude (pulse width and scan rate). During the study, each parameter was changed while the others were kept constant: pulse amplitude over the range of 30-100 mV, pulse width 30-80 ms, and scan rate 20-250 mV/s.

2.7. General procedure

Voltammetric analyses were performed in 25 ml of BR buffer. The solution was continuously stirred at 1200 rpm when accumulation potential (usually open circuit conditions) was applied for a certain time to the working electrode. At the end of accumulation period, the stirring was stopped, and after 5.0 sec rest period was allowed for the solution to become quiescent. The used drug was determined by using DPV method. Aliquots of the drug solution of 10^{-3} M were introduced into the electrolytic cell and the procedure was repeated. The voltammograms were recorded. The peak current was evaluated as the difference between each voltammogram and the background electrolyte voltammogram. All measurements were carried out at room temperature.

2.8. Preparation of urine sample

Determination of Cilostazol in spiked urine samples, 1.0 ml of urine mixed with 24 ml of buffer of the optimum pH, without treatment then transferred to the voltammetric cell, and Carry out the differential pulse voltammetric procedure as described above for the pure drug.

3. RESULTS AND DISCUSSION

Electrochemical oxidation behaviour of Cilostazol at carbon past and glassy carbon electrodes has been examined over the pH range of 2.0 to 12.0. In Britton - Robinson (BR) buffers solutions and at a scan rate of 100 mV/s. Each voltammogram exhibits one well defined anodic peak at 663 mV for CPE and 682 mV for and GCE , with no peak on the reverse scan, suggesting the irreversible nature of the electrode reaction in the cathodic scan; pointing to the irreversible nature of the oxidation process. Fig.2. shows the cyclic voltammogram of 2.5×10^{-6} M Cilostazol in Britton-Robinson buffer of pH 8.0. The cyclic voltammetric behaviour of the compound gives one oxidation process at all pH values. On the reverse sweep, no distinct reduction wave was observed, indicating that the drug is irreversibly oxidized at the GCE OR CPE.

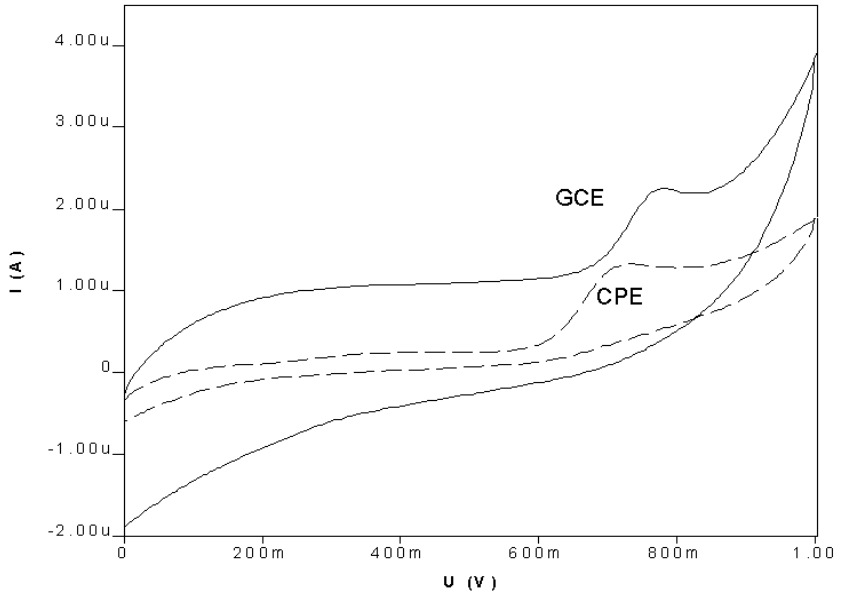

Fig. 2. Cyclic voltammograms of 2.5×10^{-6} M Cilostazol solution in BR buffer of pH 8 CPE and GCE at a scan rate 100 mV/s

3.1. Effect of pH

The pH effect on Cilostazol at carbon paste and glassy carbon electrode was studied. Fig.3. shows the plot of peak current (I_p) *vs*. pH indicating that the peak current reaches its maximum value at pH 8 at carbon paste and glassy carbon electrodes.

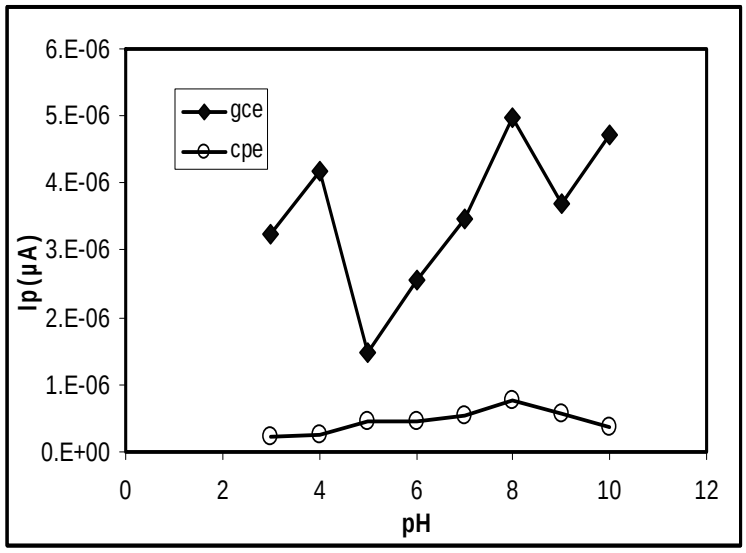

Fig. 3. Effect of pH on peak current of 2.5×10^{-6} M Cilostazol solution in BR buffer at CPE paste and GCE at a scan rate 100 mV/s

3.2. Effect of various buffers

Cyclic voltammetery experiments of 2.5×10^{-6} M Cilostazol solution in BR and borate Buffer solutions of pH 8.0 at glassy carbon electrodes were carried out as shown in Fig. 4. It is obvious from the figures that BR buffer is suitable for electrochemical determination of Cilostazol.

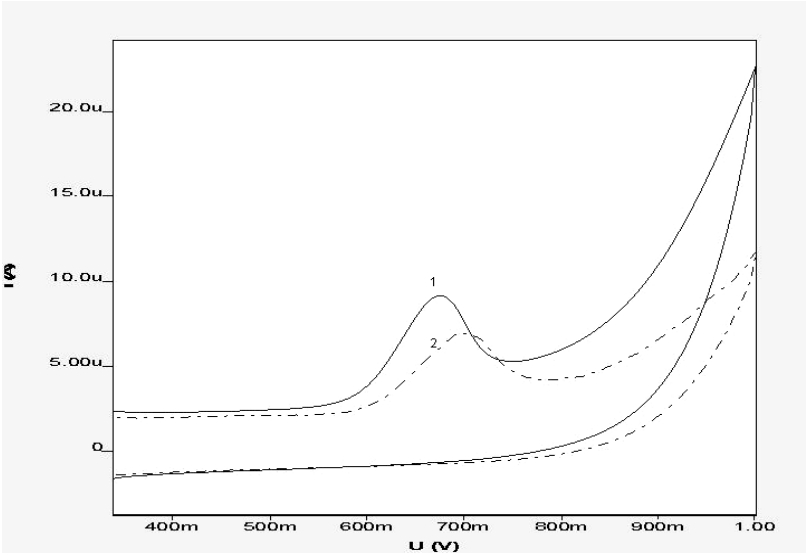

Fig. 4. Cyclic voltammograms of 2.5×10^{-6} M Cilostazole solution at GCP in BR buffer (1) and borate buffer (2) at pH 8. Scan rate100 mV/s

3.3. Effect of scan rate

The effect of the potential scan rate on the peak current (I_p) of (2.5×10^{-6} M) Cilostazol solution was evaluated log I_p and log υ over the scan range 20-200 mV/s as shown in Fig.5,6. The relation between log peak current (I_p) and log scan rate shown in Fig.7. And give this equation I_p=-5.9588+0.2258 log υ, and log I_p=- 5.5094+0.5202 log υ in case of carbon paste electrode and glassy carbon electrode, respectively. From slope in case of GCE 0.520, it indicates diffusion controlled process with some adsorption character and the slope of 0.2258 is close to the theoretically expected value of 0.50 for a diffusion controlled process [20]. The peak potential moves to more positive potentials as the scan rate increases, which confirms the irreversibility of the process. An increase in scan rate triggers, an increase of the peak current, with this increase being proportional to ν. The presentation of log I_p *vs*. log ν results in a straight line with a slope of 0.5202, illustrating that the electrode process is an adsorption controlled process [21-23].

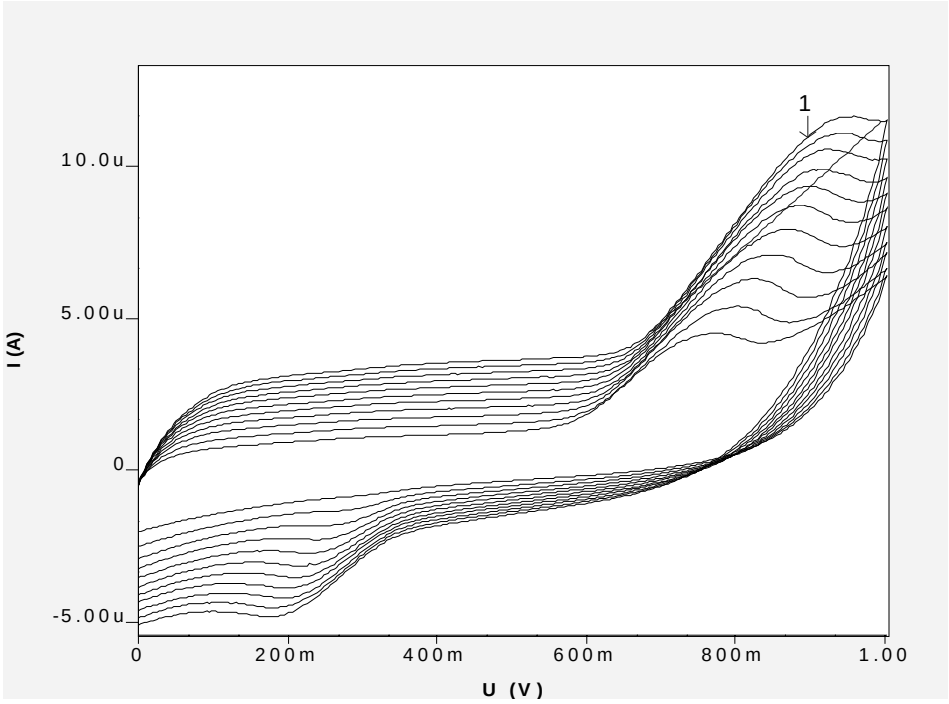

Fig. 5. Scan rate (υ) in BR buffer of pH 8 of 2.5×10^{-5} M Cilostazol solution at GCE

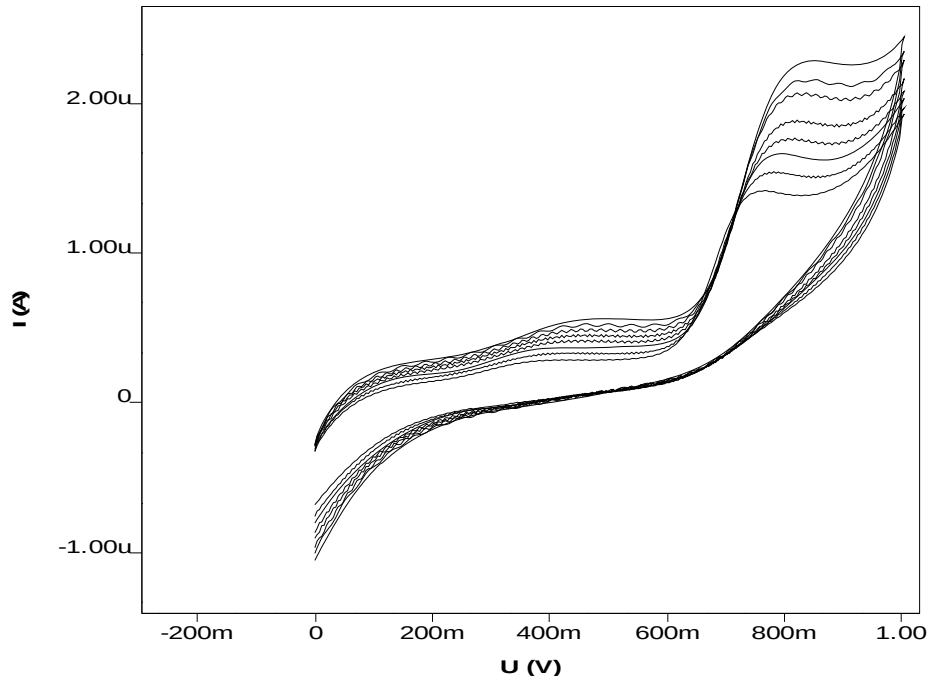

Fig. 6. Scan rate (υ) in BR buffer of pH 8 of 2.5×10^{-5} M Cilostazol solution at CPE

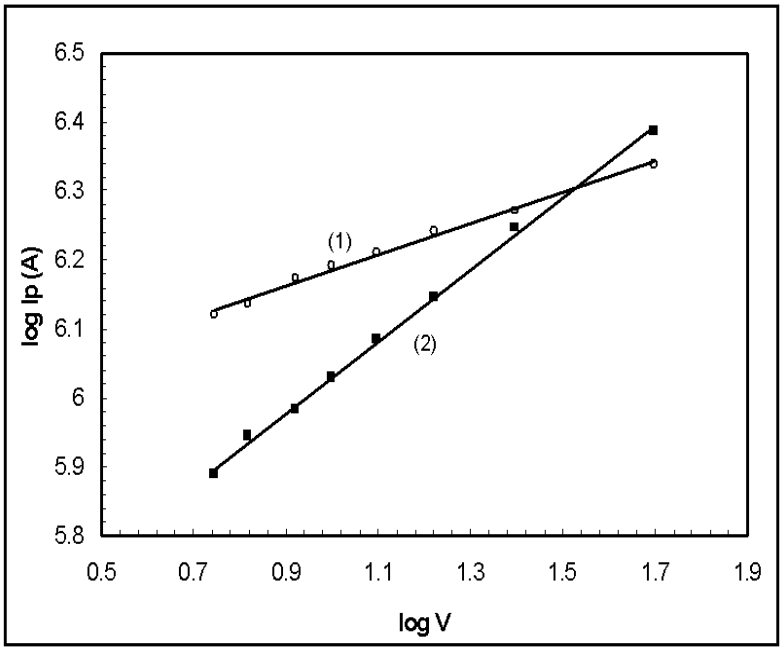

Fig. 7. Anodic peak current response of 2.5×10^{-5} M Cilostazole solution as a function of scan rate (υ) in BR buffer of pH 8 at CPE (1) and GCE (2)

3.4. Effect of accumulation time

The effect of accumulation time on the anodic peak current of 2.5×10^{-6} M Cilostazole solution at pH 8 was studied for carbon paste electrode at open circuit conditions and the results were shown in Fig. 6. It is concluded that increasing peak currents were obtained up to accumulation time 30 sec. Hence 30 s is chosen as the optimum accumulation time, From Fig 7. Slope in case of GCE 0.520, it indicates diffusion controlled process with some adsorption character hence undergoes the effect of a adsorption time as in Fig. 8 it was found that 30 s chosen as the optimum accumulation time for GCE.

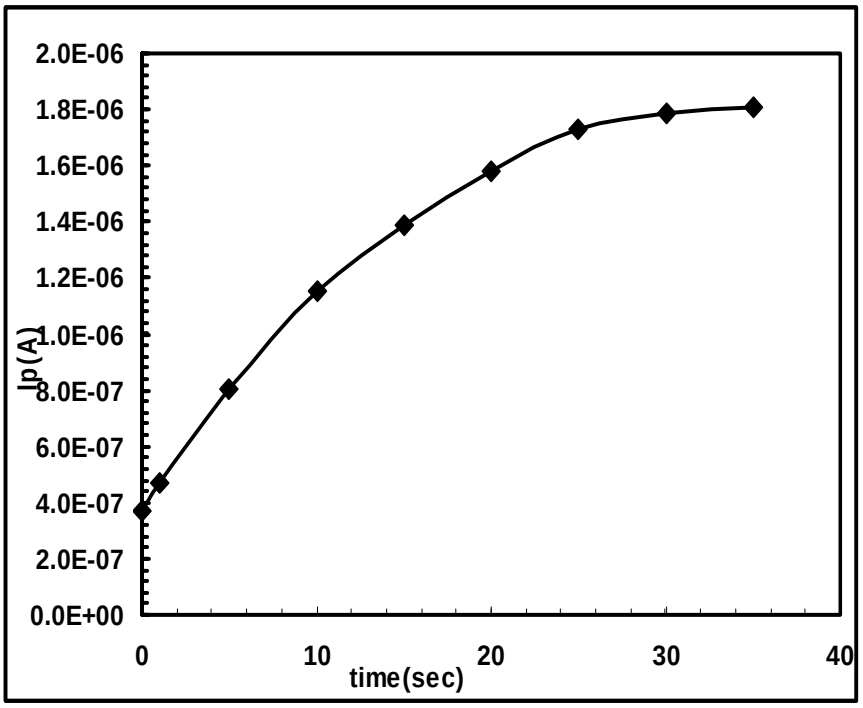

Fig. 8. Effect of accumulation time on the peak current 2.5×10^{-6} M Cilostazole solution in BR buffer of pH 8 at GCE

3.5. Determination of Cilostazole in the pure form

Cilostazole was electro oxidize at carbon paste and glassy carbon electrode in the working potential ranges 0–1000 mV. However, Fig 9, 11 shows that Cilostazole showed a well oxidation peak 663 and 682 mV potential at carbon paste and glassy carbon electrode in differential pulse voltammetry. The peak current of the oxidation peak increased with the increasing in concentration of Cilostazole. Analytical method was developed involving differential pulse voltammetry for the determination of the drug under investigation. The peak current shows a linear dependence with Cilostazole concentration between 4.0×10^{-7}–

6.40×10^{-6}M carbon paste electrode and 4×10^{-7}–7.7×10^{-6} M at glassy carbon electrode, respectively. The calibration plots were described in Fig 10, 12 by the following equations:
I_p(µA)=0.038 C (µM)+4E-08 (Correlation coefficient)=0.9932 carbon past electrode
I_p(µA)=0.0785 C (µM)-1E-08 (Correlation coefficient) = 0.9963 glassy carbon electrode

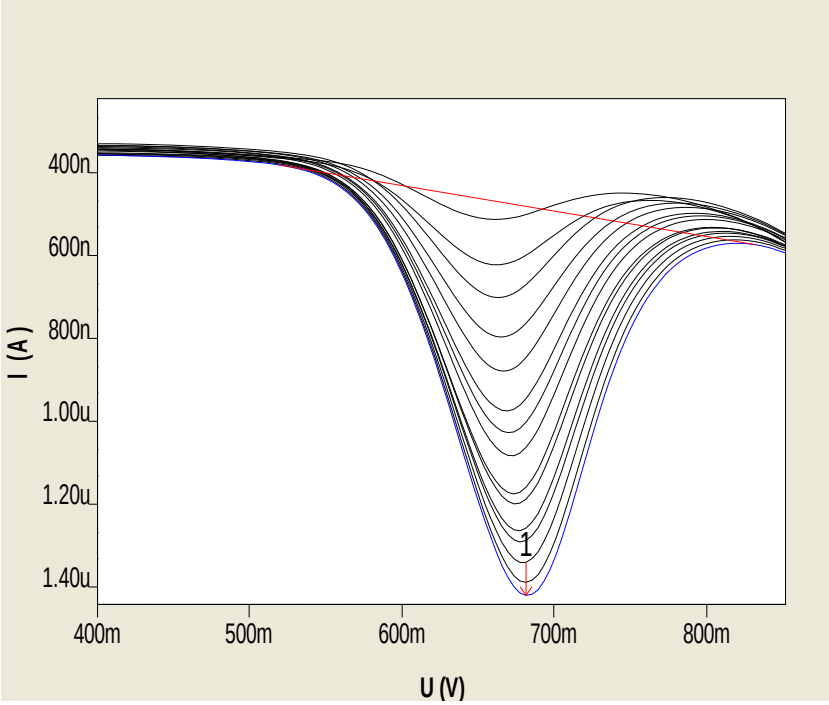

Fig. 9. Differential pulse voltammograms recorded Cilostazol a(4.0×10^{-7} M), b(9.0×10^{-7} M), c(1.4×10^{-6} M), d(1.9×10^{-6} M), e(2.4×10^{-6} M), f(2.9×10^{-6} M), g(3.4×10^{-6} M), h(3.9×10^{-6} M), i(4.4×10^{-6} M), j(4.9×10^{-6} M), k(5.4×10^{-6} M), l(5.9×10^{-6} M), m(6.4×10^{-6} M),n(6.9×10^{-6} M) and o(7.7×10^{-6} M), in BR buffer of pH 8 at Glassy carbon electrode

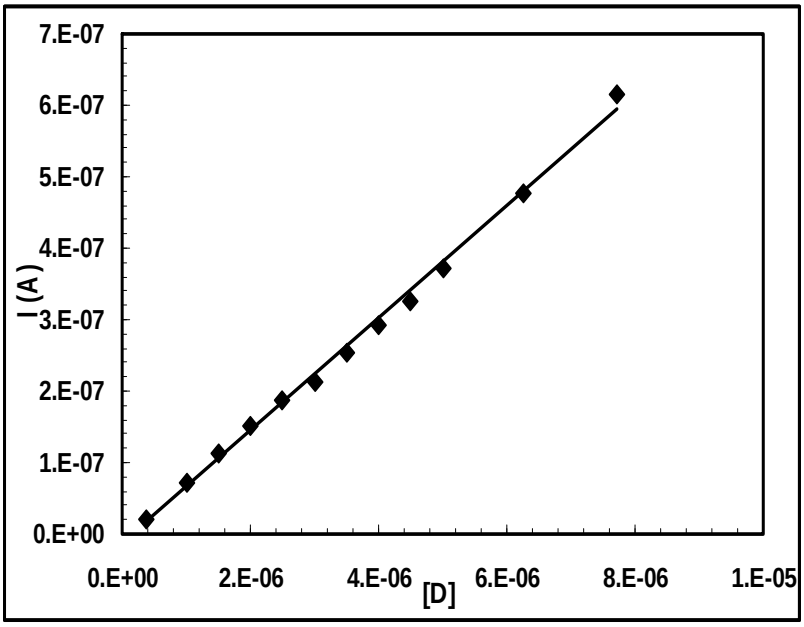

Fig. 10. Linear calibration curves of anodic peak currents *vs*. concentration in the range 4×10^{-7}–7.7×10^{-6} M. of Cilostazol at Glassy carbon electrode

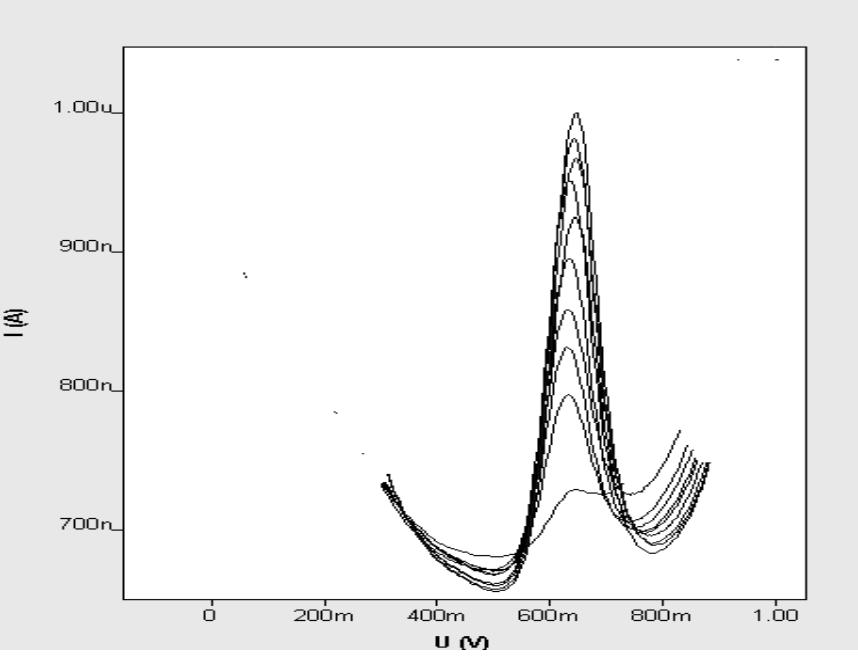

Fig.11. Differential pulse voltammograms recorded Cilostazol a(4.0×10^{-7} M), b(1.15×10^{-6} M),c(1.9×10^{-6} M),d(2.65×10^{-6} M),e(3.4×10^{-6} M),f(4.15×10^{-6} M),g(4.9×10^{-6} M),h(5.65×10^{-6} M), and i (6.4×10^{-6} M) in BR buffer of pH 8 at carbon past electrode

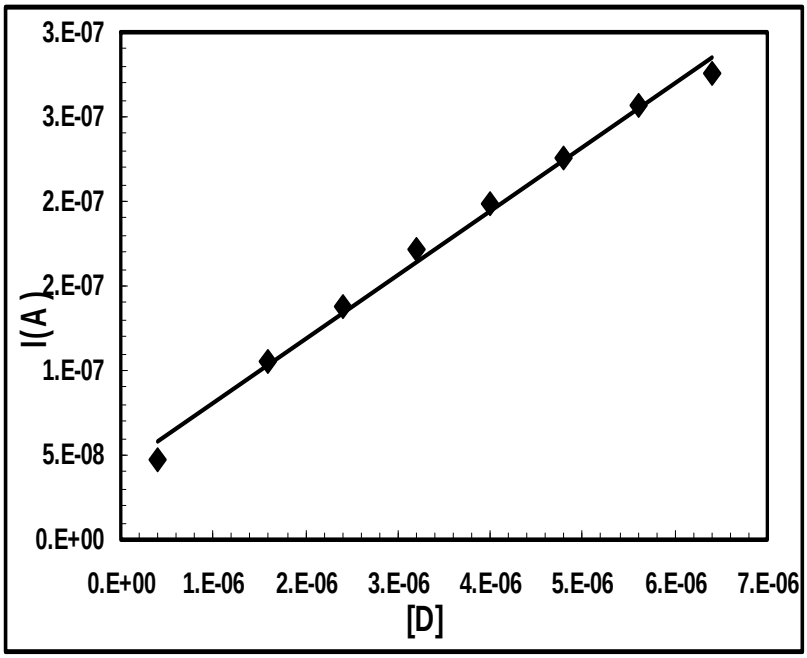

Fig. 12. Linear calibration curves of anodic peak currents *vs*. concentration in the range 4.0×10^{-7}–6.40×10^{-6} M of Cilostazol at carbon past electrode

3.6. Analytical applications of commercial tablets

The applicability of the proposed method for determination of pharmaceutical form various Cilostazol containing tablets were examined for estimating Cilostazol content present in commercial dosage were statistically them. Differential pulse was recorded under exactly identical a condition that was employed while recording for plotting calibration plot. The proposed method was successfully applied to the direct determination of pletal, sedotazole, infarca, Claudol, claudizol, without interference from some common excipients used in pharmaceutical preparations. The linearity range was 4.0×10^{-7}-3.2×10^{-6} M with mean recovery of 100. 30% and mean relative standard deviation of 0.726% in case of carbon paste electrode. For glassy carbon electrode the linearity range was 4.0×10^{-7}-2.8×10^{-6} M with mean recovery of 100.27% and mean relative standard deviation of 0.677%.

Table 1. Statistical parameters of the pharmaceutical dosage forms from assay of the investigated drug by the proposed DPV method and official method

Sample	[Drug] μgL^{-1} taken	Proposed method ±%RSD, n=5 (GCE)	Proposed method ±%RSD, n=5 (CPE)	Official method ±%RSD, n=5	F-test		T-test	
					(GCE)	(CPE)	(GCE)	(CPE)
pletal	50	100.11±0.9	100.2±0.4	100.1±2.1	1.5	1.66	2.33	2.11
	100	99.82 ± 1.2	100.11±1.1	99.52±0.97	1.72	1.70	2.1	2.21
sedotazole	50	100.01±2.1	100.05±1.04	99.41±1.42	1.33	1.47	2.1	2.08
infarca	50	99.3±1.1	100.07±0.81	99.95±1.14	1.44	1.53	2.07	2.24
Claudol	100	100.5±1.16	100.2±1.2	99.65±1.55	1.87	1.61	2.48	2.31
claudizol	100	99.98±1.21	99.88±0.45	100.18±1.54	1.88	1.32	2.22	2.36

3.7. Recovery test

Recovery test of Cilostazol was carried out by spiking of Cilostazol with highly diluted urine samples. The urine sample was spiked with different amount of Cilostazol. The results obtained are listed in Table 2. Recoveries have been found to lie in the range 99.43–100.5%, 99.00-103% for GCE and CPE respectively with a relative standard deviation of 5.1%. , 4.1% at GCE and CPE respectively.

Table 2. Recovery data observed for spiked Cilostazol in highly diluted urine sample

Spiked (m mol l^{-1})	Detected (m mol l^{-1})		Recovery (%)	
	GCE	CPE	GCE	CPE
0.20	0.199	0.202	99.50	101.00
0.25	0.253	0.489	101.20	195.60
0.30	0.31	0.298	103.33	99.33
0.35	0.348	0.351	99.43	100.29
0.40	0.402	0.401	100.50	100.25
0.50	0.497	0.499	99.40	99.80

3.8. Comparison between GCE and CPE

A comparison between the characterization of two proposed GCE and CPE was carried out in Table 3.

Table 3. Comparison between GCE and CPE

Parameters	DPV at CPE	
	CPE	GCE
pH	8.0	8.0
Calibration curve limits	4.0×10^{-7}–6.40×10^{-6} M	4×10^{-7}–7.7×10^{-6} M
Regression equation	$I_p(\mu A)=0.038C\ (\mu M)+4E-08$	$I_p(\mu A) = 0.0785\ C\ (\mu M)\ -1E-08$
Correlation coefficient (r)	0.9963	0.9932
Error*%	0.2023	0.1273
RSD %	5.1	4.1

4. CONCLUSION

The proposed electroanalytical method described here showed a well peak at ~ 663 and ~682 mV potential at carbon paste and glassy carbon electrode in differential pulse voltammetry. Hence, the proposed differential pulse voltammetry method could be used successfully to determine Cilostazol in pure form, pharmaceutical forms and urine it is enables, simple, low cost, sensitive, selective, accurate and precise. The sensitively and selectivity of the procedure could be improved by the preconcentration of the drug from large sample volumes under the optimum experimental condition. The method developed also showed good ability to quantify drug contents in tablets with reliable accuracy and can also be used to determine unmetabolized drug in urine samples.

REFERENCES

[1] S. Shintani, K. Watanabe, K. Kawamura, T. Mori, T. Tani, Y. Toba, H. Sasabe, N . Nakagiri, O. Hongoh, S. Fujita, T. Aogi, Y. Kido, M. Umezzato, M. Ishikawa, and T. Hiyama, Arzneim-Forsch./Drug Res. 35 (1985) 1163.

[2] H. Akiyama, S. Kudo, and S. Shimizu, Arzneim-Forsch./Drug Res. 35 (1985) 1133.

[3] H. Akiyama, S. Kudo, and S. Shimizu, J. Chromatogr. Biomed. Appl. 338 (1985) 456.

[4] P. N. V. Tata, C. J. Fu, N. J. Browder, P. C. Chow, and S. L. Bramer, J. Pharm. Biomed. Anal. 18 (1998) 441.

[5] H. Akiyama, S. Kudo, M. Odami, and T. Shimizu, J. Chromatogr. 338 (1985) 456.

[6] A. S. Fayed, M. A. Shehata, A. Ashour, N. Y. Hassan, and S. A. Weshahy, J. Pharm. Biomed. Anal. 45 (2007) 407.
[7] C. H. J. Fu, P. N. V. Tata, K. Okada , H. Akiyama, and S. L. Bramer, J. Chromatogr. B 728 (1999) 251.
[8] P. N. V. Tata, C. H. J. Fu, and S. L. Bramer, J. Pharm. Biomed. Anal. 24 (2001) 381.
[9] C. J. Fu, P. N. Tata, K. Okada, H. Akiyama, and S. L. Bramer, J. Chromatogr. B Biomed. Appl. 728 (1999) 251.
[10] S. L. Bramer, P. N. V. Tata, S. S. Vengurlekar, and J. H. Brisson, J. Pharm. Biomed. Anal. 26 (2001) 637.
[11] A. S. Fayed, M. A. Shehata, A. Ashour, N. Y. Hassan, and S. A Weshahy, J. Pharm. Biomed. Anal. 45 (2007) 407.
[12] B. Uslu, and S. A. Ozkan. Anal. Chim. Acta 462 (2002) 49.
[13] A. Radi, and Z. El-Sherif. Talanta 58 (2002) 319.
[14] B. Nigovie, and B. Simmunie, J. Pharm. Biomed. Anal. 32 (2003) 197.
[15] I. Tapsoba, J. Belgaied, and K. Boujlel, J. Pharm. Biomed. Anal. 38 (2005) 162.
[16] A. Radi, Talanta 65 (2005) 271 .
[17] A. Ambrosi, R. Antiochia, L. Campanella, R. Dragone, and I. Lavagnini. J. Hazard. Mater. 122 (2005) 219.
[18] Y. Ni, Y. wang, and S. Kokot, Talanta 69 (2006) 216.
[19] T. S. Britton, and R. A. Robinson, J. Chem. Soc. 458 (1931) 287.
[20] D. K. Gosser, Cyclic Voltammetry Simulation and Analysis of Reaction Mechanism, VSH, New York (1994).
[21] E. Laviron, J. Electroanal. Chem. 112 (1980) 1.
[22] D. K. Gosser, Cyclic voltammetry, VCH, New York (1994).
[23] A. Radi, Anal. Bioanal. Chem. 378 (2004) 822.