Int. J. Electrochem. Sci., 3 **(2008)** 756 - 767

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Electrochemical Behavior of an Anti-Cancer Drug at Glassy Carbon Electrode and its Determination in Pharmaceutical Formulations

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Received: 11 March 2008 / *Accepted:* 12 April 2008 / *Published:* 29 May 2008

Capecitabine (CPT) is an anticancer prodrug of 5-fluorouracil (5-FU) used in the treatment of colorectal and breast cancer. The electrochemical behavior of CPT at glassy carbon electrode was investigated by cyclic, linear sweep and differential pulse voltammetry (DPV). A well defined reduction peak of CPT at 4 x 10^{-5} M was observed in Britton-Robinson (BR) buffer of pH 2.5. The reduction process was observed to be irreversible over the pH range of 1-7. The influence of different electrolytes, pH, scan rate and concentration of the drug on cathodic peak was studied. The probable reaction mechanism involved in the reduction of CPT was also proposed. A DPV method with good precision and accuracy was developed for the determination of CPT in pharmaceutical formulations. The peak currents were found to be linearly dependent on the concentration range of 8 x 10^{-7} - 5 x 10^{-5} M CPT. The limit of detection (LOD) and limit of quantification (LOQ) were noticed to be 1.13 x 10^{-7} and 3.78 x 10^{-7} M, respectively.

Keywords: Capecitabine; electrochemical studies; Differential pulse voltammetry; Pharmaceutical formulation

1. INTRODUCTION

Capacetabine (CPT), chemically N^4-pentoxycarbonyl-5'-deoxy-5-fluorocytidine, is a prodrug of 5-fluorouracil (5-FU). 5-FU is widely used as an anticancer agent in the chemotherapy of solid tumors, but its efficacy is limited by dihydropyrimidine dehydrogenase catalyzed formation of dihydro-5-fluorouracil. Since it lacks selectivity toward tumor cells, 5-FU exhibits significant toxicity [1,2]. Some of the prodrugs of 5-FU show adverse effects like diarrhea after oral and intravenous administration [3,4]. CPT was developed to reduce such adverse effects while improving the

selectivity toward tumors [5,6]. After oral administration, CPT is rapidly and extensively absorbed (greater than 80%) from the gastrointestinal track and then converted to its metabolites such that side effects like diarrhea are much less likely to occur with its use [1,5]. CPT is an oral tumor-selective fluoropyrimidine carbamate approved in the treatment of colorectal and breast cancer [7,8]. CPT is sequentially metabolized to 5-FU by carboxylesterase, cytidine deaminase and thymidine phosphorylase which show relatively specific organ expression [9].

Few analytical methods *viz.,* HPLC, LC–MS and LC–MS/MS have been reported for the assay of CPT [10-16] in biological samples. These methods require long analysis time, elaborate extraction and purification steps or on-line sample extraction. More over, these methods do not report the analysis of CPT in formulations. Since, LC-MS and LC–MS/MS are relatively costly, we thought of developing an analytical method for the assay of CPT in its formulations.

In recent years the electrochemical techniques have led to the advancement in the field of analysis because of their sensitivity, low cost and relatively short analysis time when compared with other techniques. Additional application of electroanalytical techniques includes the determination of reaction mechanisms. Redox properties of a drug can give insights into its metabolic fate or its *in vivo* redox processes or pharmaceutical activity [17-19]. Critical literature survey revealed that no attempt has been made to investigate the electrochemical behavior of CPT and to determine it in pharmaceutical formulation by voltammetry. In view of this, we have developed a simple electrochemical method for the assay of CPT in formulations. The present paper describes the investigations on the electrochemical behavior of CPT at glassy carbon electrode for the first time and development of a DPV method for its determination in formulations.

2. EXPERIMENTAL PART

2.1. Apparatus

The voltammetry experiments were performed with CH Instruments, USA (Model 1110A, Version 4.01). A three-electrode system consisting of a glassy carbon electrode (3 mm diameter) as the working electrode, a Ag/AgCl (3 M KCl) reference electrode and a platinum wire as the auxiliary electrode was used. In order to provide a reproducible active surface and to improve the sensitivity and resolution of the voltammetric peaks, the glassy carbon electrode was polished to a mirror finish with 0.3 micron alumina on a smooth polishing cloth and then rinsed with methanol and double distilled water prior to each electrochemical measurements. The electrode cleaning procedure requires less than 3 min. All the solutions examined by electrochemical techniques were purged for 10 min with water-saturated nitrogen. All measurements were carried out at room temperature (24 ^{0}C).

The pH measurements were made on a Schott Gerate pH meter CG 804.

DPV conditions maintained were- pulse amplitude, 50 mV; pulse width, 30 ms and scan rate, 20 mV/s.

2.2. Reagents

A stock solution of CPT (1 mM) was prepared in water and stored in a refrigerator at 4 ^{o}C. Working solutions of the drug were prepared daily by diluting the stock solution with a selected supporting electrolyte. All other chemicals used in this investigation were of analytical grade. All solutions were prepared in doubly distilled water. The H_2SO_4 solution (pH 1-2; 0.1 M), Britton-Robinson buffer (pH 2-10; 0.04 M) and acetate buffer (pH 3.6-5.6; 0.2 M) were used as the supporting electrolytes.

2.3. Assay of Tablets

The tablets of CPT were obtained from local commercial sources. Ten tablets were weighed accurately and ground to a fine powder. A portion of the powder equivalent to 1 mM CPT was transferred to a 100 ml volumetric flask and completed to volume with distilled water and sonicated for 15 min to effect complete dissolution. The sample from the clear supernatant liquor was withdrawn and quantitatively diluted with the selected supporting electrolyte. The content of the drug in tablet was determined referring to the calibration graph or regression equation.

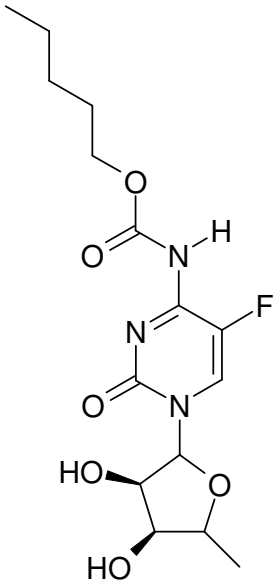

Figure 1. The molecular structure of CPT

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of CPT

Fig. 1 shows molecular structure of CPT. Cyclic and linear sweep voltammetric techniques were employed to understand the electrochemical process of CPT occurring at glassy carbon electrode.

The cyclic voltammogram of CPT at 4 x 10^{-5} M in BR buffer of pH 2.5 showed a well-defined cathodic peak (Fig. 2). No peak was observed in the reverse scan suggesting that the reduction process is an irreversible one. Inset in Fig. 2 shows the linear sweep voltammogram of CPT in BR buffer of pH 2.5.

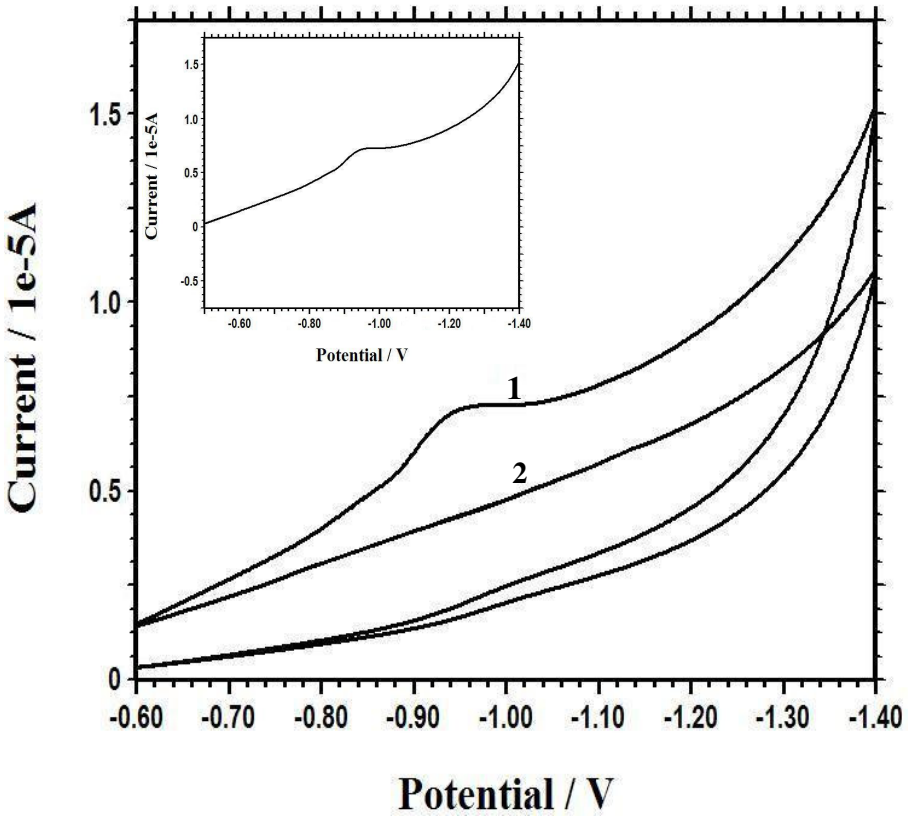

Figure 2. Cyclic voltammograms of 4.0 x 10^{-5} M CPT in BR buffer at pH 2.5 (1) and blank (2). Inset: Linear sweep voltammogram. Scan rate maintained at 100 mV/s

The effects of scan rate, different electrolytes (H_2SO_4, BR buffer and acetate buffer), pH (1-10) and concentration of the drug on the peak current and peak potential were investigated. The best curve and the highest current were obtained in BR buffer of pH 2.5 at a scan rate of 100 mV/s. The multisweep cyclic voltammograms of CPT at 4 x 10^{-5} M in BR buffer of pH 2.5 at a scan rate 100 mV/s are shown in Fig. 3. The decrease in peak current with negative shift in peak potential with succeeding potential scans suggested the formation of adsorbed species on the electrode surface. A linear dependence of the peak intensity upon the square root of scan rate was found in the range of 5-200 mV/s for 4 x 10^{-5} M CPT, which is the typical of diffusion controlled current [20]. Fig. 4 shows cyclic voltammograms of CPT (4 x 10^{-5} M) at different scan rates in BR buffer of pH 2.5. The corresponding equation is shown below:

$$i_p(\mu A) = 0.37\ \nu^{1/2}\ (mV/s) + 0.546,\ \ r = 0.9975$$

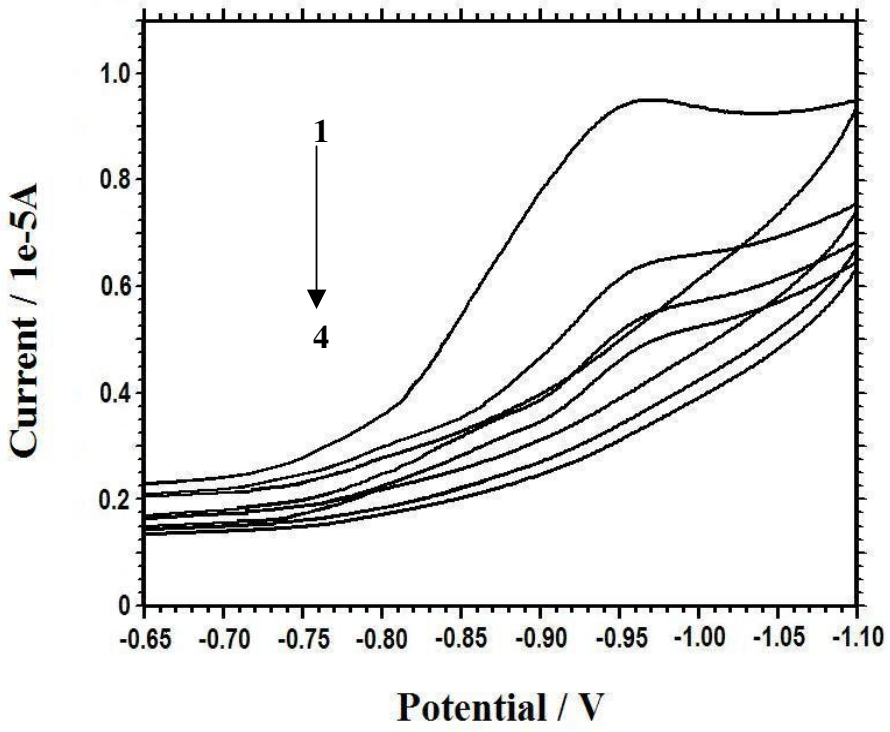

Figure 3. Successive cyclic voltammograms for 4.0 x 10^{-5} M CPT on glassy carbon electrode in BR buffer at pH 2.5. Scan rate, 150 mV/s. 1 to 4 indicate first scan to fourth scan

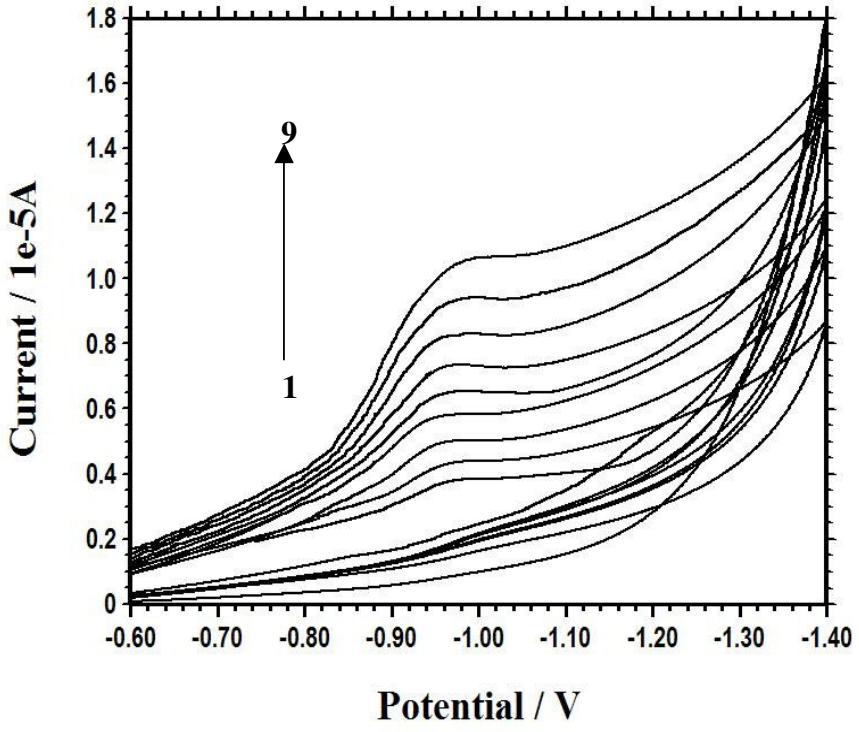

Figure 4. Cyclic voltammograms of 4.0 x 10^{-5} M CPT at different scan rates 5 (1) 20 (2) 50 (3) 80 (4) 100 (5) 130 (6) 150 (7) 170 (8) and 200 (9) mV/s

With the scan rate of 5-200 mV/s, the plot of logarithm of peak current (log i_p) *versus* logarithm of scan rate (log ν) gave a slope of 0.46 which is close to the theoretical value of 0.5 that is expressed for an ideal reaction for the diffusion-controlled electrode process [21]. The equation obtained is,

$$\log i_p\ (\mu A) = 0.46 \log v\ (mV/s) - 0.18,\ r = 0.9958$$

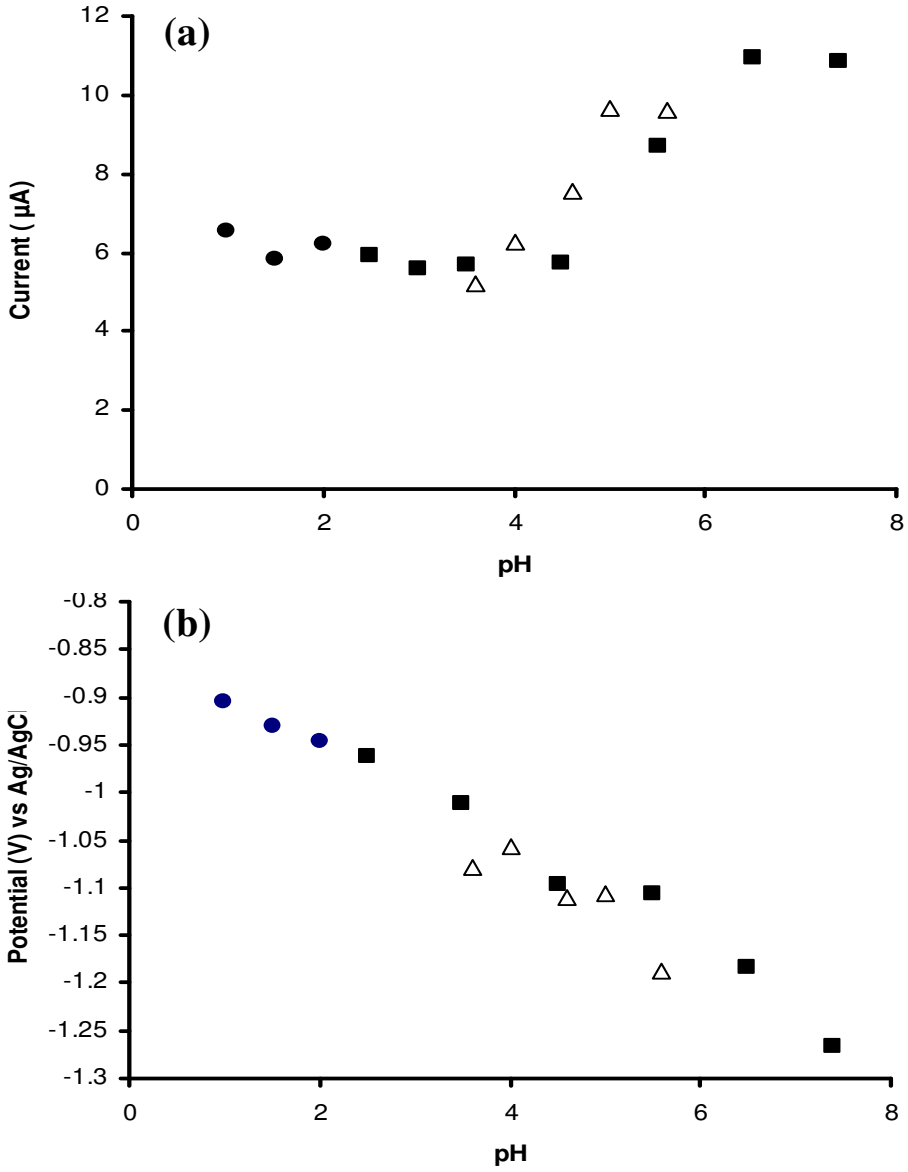

Figure 5. Effects of pH on cathodic peak current (a) and peak potential of CPT (b): CPT concentration was 4 x 10^{-5} M. 0.01, 0.03 and 0.1 M H_2SO_4 (●); 0.04 M BR buffer (■); and 0.2 M Acetate buffer (Δ).

Above the scan rate of 200 mV/s and up to 1 V/s, the same plot yielded the slope value of 0.94 which is close to the theoretical value of 1.0 that is expected for adsorption controlled electrode process [21]. The reduction of CPT was observed in the pH range of 1-7. Above the pH 7, no peak was observed probably due to the participation of H^+ in the reduction process. The pH dependence of peak current (i_p) and peak voltage (E_p) is shown in Fig. 5. It was observed that the peak potentials shifted to more negative values with increase in pH (Fig. 5). This revealed that the pH of the supporting electrolyte exerted the significant influence on the reduction of CPT at glassy carbon electrode. Further, it was evident that the reduction process was more facile at low pH values. The corresponding equation is,

$$E_p = -0.0592\ pH + 0.8098,\ r = 0.9987$$

The slope of the above plot was found to be 0.0592 indicating thereby that the number of protons participating in the electrode reaction is same as that of electrons [22]. The plot of i_p *versus* concentration showed linearity over the range of 8.3 x 10^{-6} M to 5 x 10^{-5} M (from CV) suggesting further that the electrode process was diffusion controlled [23,24].

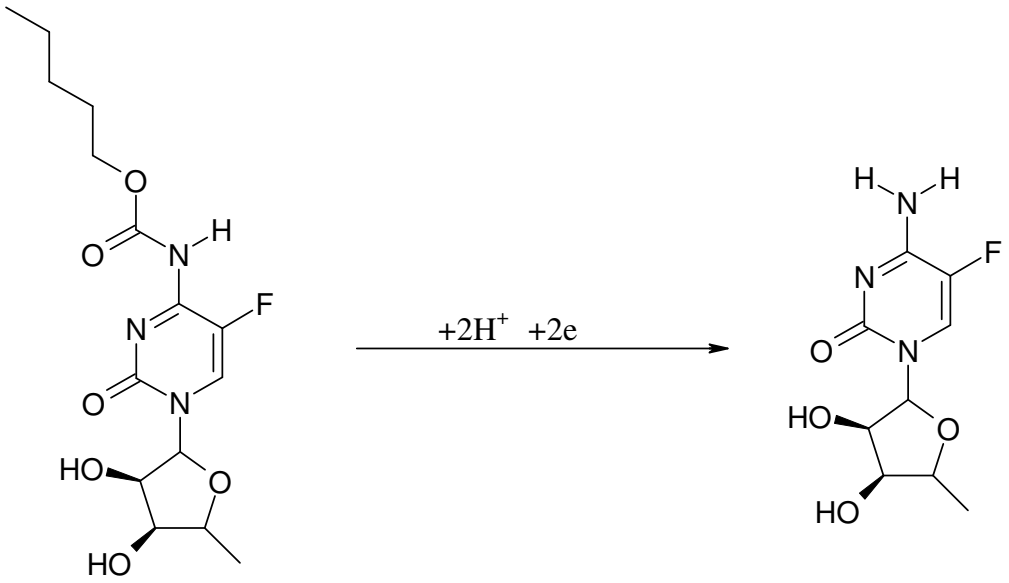

Figure 6. Probable reaction mechanism for reduction of CPT

The number of electrons involved in the reaction was calculated using the equation shown below [25].

$$i_{pa} = (2.99 \times 10^5)\ n\ (\alpha_{na})^{1/2}\ A\ D_o^{1/2}\ \nu^{1/2}\ C_o^*$$

where, n = number of electrons, A = area of the electrode, D_o = diffusion coefficient, ν = scan rate, C_o^* = concentration of electroactive species and α_{na} = transfer coefficient. The value of α_{na} can be calculated using the equation shown below [25]:

$$\Delta E_p = E_p - E_{p/2} = (47.7) / \alpha_{na}$$

where E_p is the peak potential and $E_{p/2}$ is the half wave potential. The values of n and α_{na} were found to be 2 and 0.40, respectively. This value of α_{na} revealed the total irreversibility of the electron transfer [26]. From the pH studies also, the number of electrons and protons participating in the reaction was observed to be the same.

The carbonyl group present in CPT is likely to be protonated in the reaction mixture owing to high polarity in the given stereoelectronic factors. Hence, the molecule gets reduced to free amino group by N-C bond cleavage. Further, under the considered potential limits, the 5'-deoxy-5-fluorouridine part of the CPT molecule could not be reduced. Therefore, it was believed that the reduction was taking place at other than 5'-deoxy-5-fluorouridine part of CPT. Based on the above experimental results, the probable reaction mechanism for the reduction of CPT is shown in Fig. 6.

3.2. Optimization of measurement conditions

The influence of several electrolytes (H_2SO_4, BR and acetate buffers) on the analytical signal was studied using different electroanalytical techniques. Considerably improved sensitivity can be achieved by application of differential pulse voltammetry for the determination of CPT. Sharper and well defined curves were obtained in BR buffer of pH 2.5. With increase in pH, broad and ill defined peaks were observed. Further, no peak was observed above pH 7 due to the non availability of protons for reduction. Hence, we have carried out the electrochemical studies at pH 2.5 in subsequent work.

The measurement conditions were optimized by observing the variation of the peak current with pulse amplitude, pulse width and scan rate. The best peak was observed with 50 mV pulse amplitude, 30 ms pulse width and 20 mV/s scan rate. With increasing pulse amplitude from 25 to 90 mV, the peak current increased but the peak became broader and ill defined. However, the peak current decreased as the pulse width increased from 30 to 90 ms. Further, the peak current increased linearly with the scan increment up to 20 mV/s.

3.3. Validation of the analytical procedure

Based on the electrochemical reduction of CPT at glassy carbon electrode, an analytical method, differential pulse voltammetry (DPV) was developed for the determination of CPT. The BR buffer of pH 2.5 was proved to be the best condition for its assay. The differential pulse voltammograms of CPT at different concentrations are shown in Fig. 7. In the optimized conditions, a linear relation between the peak current and the drug concentration was observed in the range of 8 x 10^{-7} to 5 x 10^{-5} M CPT. Above this concentration, loss of linearity was noticed probably due to the

adsorption of CPT on the electrode surface. Standard deviation values for the slope and intercept of the calibration curve were calculated to be 0.00135 and 0.00712, respectively. Characteristics of the calibration graph are reported in Table 1. Validation of the optimized procedure for the quantitative assay of CPT was examined via evaluation of the limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and recovery. LOD and LOQ were calculated based on the peak current using the following equations [27,28].

$$LOD = 3\ s/m;\ LOQ = 10\ s/m$$

where s is the standard deviation of the peak current (five replicates) and m is the slope of the calibration curve. The LOD and LOQ values were found to be 1.13 x 10^{-7} M and 3.78 x 10^{-7} M, respectively. Low values of LOD and LOQ confirmed the sensitivity of the proposed method. The inter-day reproducibility of the method was examined by recording voltammogrammes of 7 replicates of 5.5 x 10^{-6} M, 1.3 x 10^{-5} M, and 2.2 x 10^{-5} M. These yielded the RSD values of 1.03, 1.59 and 1.24 % respectively. Further, the RSD values for intra-day assay reproducibility at 8 x 10^{-7} M and 8.3 x 10^{-6} M solutions (n = 7) were found to be 0.98 and 1.18 %. The corresponding results are shown in Table 1. Low values of RSD revealed the good precision of the proposed DPV method for the assay of CPT.

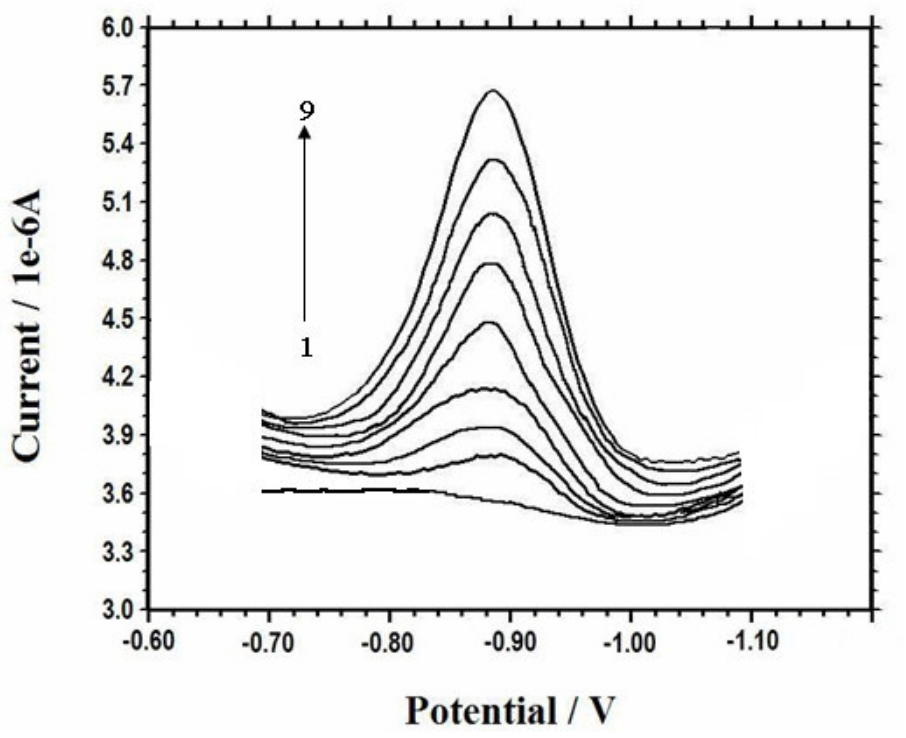

Figure 7. DPV for the increasing concentrations of CPT in BR buffer at pH 2.5 on glassy carbon electrode: pulse amplitude, 50 mV; pulse width, 30 ms and scan rate, 20 mV/s. CPT concentration (1) blank, (2) 8 x 10^{-7}, (3) 2.8 x 10^{-6}, (4) 5.5 x10^{-6}, (5) 1.1 x 10^{-5}, (6) 1.6 x 10^{-5}, (7) 2.2 x 10^{-5}, (8) 2.7 x 10^{-5} and (9) 3.3 x 10^{-5} M

Table 1. Characteristics of CPT calibration plot

	DPV
Linearity range (M)	8×10^{-7} to 5×10^{-5}
Slope of calibration graph (μAM^{-1})	5.28×10^{5}
Intercept (μA)	0.0071
Correlation Coefficient(r)	0.9982
RSD of slope	0.71
RSD of intercept	0.55
LOD (M)	1.13×10^{-7}
LOQ (M)	3.78×10^{-7}
Repeatability (RSD %)	0.98
Reproducibility (RSD %)	1.03

3.4. Determination of CPT in pharmaceutical dosages

The applicability of the proposed voltammetric method for the assay of CPT was examined by analyzing CPT in its tablets. The results of analysis of CPT in tablets are recorded in Table 2. In order to examine the precision and accuracy of the developed method,, recovery studies were carried out by standard addition method. For this, known quantities of pure CPT were mixed with definite amounts of pre-analyzed formulations of the drug and the mixtures were analyzed as before. The total amount of the drug was then determined and the amount of the added drug was calculated by difference. The results of recovery studies were found to be quantitative (99.44-101.35 %). The effects of excipients *viz*., talc, glucose, starch, lactose, dextrose, gum acacia and magnesium stearate in the determination of CPT were investigated. It was noticed that none of these interfered in the determination at the levels normally found in dosage forms.

Table 2. Assay results of CPT tablets by DPV and mean recoveries

Tablet	Capiibine [a]	Captabin [b]
Labeled claim (mg)	500	500
Amount found (mg)	501.3	498.4
RSD (%)	0.89	0.95
Amount of pure drug added to tablet solution (mg)	20	20
Amount found (mg)	20.27	19.89
Recovered (%)	101.35	99.44
RSD (%)	0.91	1.01

[a] Marketed by- Dr Reddy's Laboratories Ltd., India
[b] Marketed by- M/s Shanta Biotechnics Pvt. Ltd., India

4. CONCLUSIONS

The electrochemical behavior of CPT at glassy carbon electrode was established and studied for the first time. The electrochemical process is irreversible and pH dependent. The DPV procedure

provides a convenient and efficient method for the assay of CPT in tablets. The proposed method is rapid, requiring less than 3 min to run a sample and do not include time-consuming steps. By the proposed method, as low as 1.13 x 10^{-7} M of CPT can be accurately determined with sufficiently good precision and accuracy. The simplicity, sensitivity, selectivity and low cost of analysis are the main advantages of developed method.

ACKNOWLEDGEMENTS
We are thankful to the authorities of the Karnatak University, Dharwad, for providing the necessary facilities.

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