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Voltammetric Analysis of Clopidogrel Bisulphate in Pure Form and Pharmaceutical Formulations Using Hanging Mercury Drop Electrode

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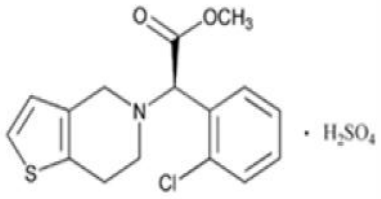
Abstract

Electroreduction and adsorption of clopidogrel (CLP) as bisulphate in pure and pharmaceutical dosage forms using differential pulse voltammetry (DPV) and differential pulse adsorptive stripping voltammetry (DPAdSV) at hanging mercury drop electrode (HMDE) has been studied. The reduction peak potential (E_p) of clopidogrel bisulphate (CLPB) using DPV was between the range -1233 to -1246 mV (versus Ag/AgCl), at pH 6.5. Linear calibration graph were in the concentration ranges of 0.060-2.000 µmol.L^{-1} (25.188-839.600 ng.mL^{-1}) with relative standard deviations did not exceed 2.8% for the concentrations of CLPB (25.188 ng.mL^{-1}). E_p was between -1225 to -1325 mV (versus Ag/AgCl) at pH 6.5 for determination of CLPB using DPAdSV. Linear calibration graphs at accumulation potential (E_{acc}) -600 mV, accumulation time (t_{acc}) 80 s and 160 s, were of 0.060-1.500 µmol.L^{-1} (16.792-629.700 ng.mL^{-1}) and 0.0050-0.300 µmol.L^{-1} (2.099-125.940 ng.mL^{-1}) with relative standard deviations did not exceed 2.6% and 3.2% for the concentrations of CLPB (16.79 and 2.099 ng.mL^{-1}), respectively. It was found that the use of DPAdSV has increased sensitivity 1.5 and 12 times at t_{acc} 80 s and 160 s, respectively. These methods give good results for the determination of CLPB in pure and different dosage forms.

Keywords: Differential pulse voltammetry, Differential pulse adsorptive stripping voltammetry, hanging mercury drop electrode, Clopidogrel bisulphate.

Introduction

Clopidogrel bisulfate is white or almost white powder. It is freely soluble in water and in methanol, practically insoluble in cyclohexane. Clopidogrel bisulphate (CLPB), methyl(+)-(s)-α-(o-chlorophenyl) 6,7-dihydrothieno (3, 2-c) pyridine-5(4H)- acetate bisulphate, is a new antiplatelet agent, and it is similar to ticlopidine in chemical structure (see scheme 1), its molecular weight of 419.8 and 321.8 g/mol of CLPB and CLP, respectively [1-3].



Scheme 1
Chemical structure of clopidogrel bisulphate (CLPB)

Many methods were developed for the analysis of CLPB in different combinations which including potentiometric method [4-6] and voltammetry [7-17], spectrophotometry [18-21], and high performance liquid chromatography [22-25].

The electroanalytical behaviour of clopidogrel bisulfate, an antithrombotic drug, was investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) using a glassy carbon electrode (GCE). The anodic oxidation of clopidogrel bisulfate was investigated with a GCE to determine the oxidation conditions. The voltammograms of solutions having various concentrations of clopidogrel were recorded. The oxidation of clopidogrel bisulfate was found to be diffusion-controlled over a concentration range of 0.08-1.0 mM in pH 3.7 acetate buffer by an optimized DPV technique [14].

Clopidogrel was analyzed using functionalized multi-walled carbon nanotubes and CdSe quantum dots modified glassy carbon electrode. The dependence of intensities of currents and potential on pH, concentration, scan rate, nature of the buffer was further investigated. For the analytical application, adsorptive stripping differential pulse voltammetric technique was used and all operational parameters were optimized. The plot of peak current of CLP concentration consisted of a linear segment in the range between 2.0×10^{-6} to 4.0×10^{-5} M. It was applied to the determination of CLP in serum samples with linear range of 2.5×10^{-6}-1.5×10^{-5} M. Determination of CLP in tablet dosage forms Atervix® was also proposed with acceptable recovery values [15].

A highly sensitive electrochemical sensor has been fabricated with silver nanoparticles embedded chitosan-carbon nanotube hybrid composite (AgChit-CNT) as sensor interface for detection of the important anti-platelet CLP drug. Electrochemical responses of the fabricated AgChit-CNT nanocomposite electrode for the determination of CLP have been examined by cyclic voltammetry and electrochemical impedance spectroscopy. Electrochemical determination of CLP was investigated by differential pulse voltammetry (DPV) and amperometric analysis under optimized conditions [16].

Differential pulse polarographic analysis (DPPA) of CLPB in pure and pharmaceutical dosage forms using drop mercury electrode (DME) and static mercury drop electrode have been studied. Various parameters (electrolyte, pH, pulse time, pulse amplitude, etc.) affecting on the CLPB determination were examined. The best definition of the analytical signals was found in sodium acetate 0.02 M buffer at pH 8.0, and in 0.02 M of H_3PO_4 -Na_2HPO_4 buffer at pH 8.5. Under the optimum conditions, linear calibration graphs, $Ip=f(C_{CLP})$, were obtained in the concentration ranges of 0.5-30 µM (0.1609-9.6546 $\mu g.mL^{-1}$ of CLP), and in the concentration ranges of 0.25-30 µM (0.08055-9.6546 $\mu g.mL^{-1}$) for the two methods, at -1275 to -1305 mV and -1278 to -1312 mV (versus Ag/AgCl) with percent relative standard deviations (RSD%) did not exceed 2.9% and 2.2% for the concentration 0.1609 $\mu g.mL^{-1}$ and 0.0805 $\mu g.mL^{-1}$ of CLP, respectively [26-27].

In the present work, DPV and DPAdSV analyses for determination of CLPB in pure form and pharmaceutical formulations using a HMDE were applied.

Experimental

Reagents

Working reference standard of clopidogrel (99.2%) was supplied by D.K. Pharmachem Pvt. Ltd INDIA, (Mfg.12-2018, Exp. 11-2021). Lithium perchlorate trihydrate, di-Sodium hydrogen phosphate dodecahydrate, Sodium chloride, Sodium hydroxid, Perchloric acid (70%), ortho-Phosphoric acid (85%), Acetic acid (100%), Boric acid (100%) were of GR for analysis purchased from MERCK.

Materials and Methods

A Metrohm 746 VA processor, A Metrohm 747 VA stand with a hanging mercury drop electrode (HMDE) as a working electrode, an auxiliary platinum electrode and a reference electrode, double junction type, (Ag/AgCl) saturated with a 3.0 M KCl solution and the three-electrode cell were used. All measurements were done at room temperature 25±5^{o}C. Highly pure nitrogen gas (99.999 %) was used for de-oxygenation. pH meter from Radiometer company model ion check was used for the studying and monitoring the pH effects. The diluter pipette model DIP-1 (Shimadzu), having 100 µL sample syringe and five continuously adjustable pipettes covering a volume range from 10 to 5000 µL (model PIPTMAN P, GILSON), were used for preparation of the experimental solutions. An ultrasonic processor model Power Sonic 405 was used to sonicate the sample solutions. Electronic balance (Sartorius-2474; d=0.01 mg) was used for weighing the samples.

A commercial formulations (as tablets) were used for the analysis of CLPB by using DVP and DPAdSV with HMDE electrode. The pharmaceutical formulations were subjected to the analytical procedures:

(1) **Pharma Grel,** F.C. Tablet, PHARMASYR, Damascus–SYRIA, each tablet contains: 75 mg of CLP (Exp. 08.2022).

(2) **Plaraz,** F.C. Tablet, AL–RAZI, Aleppo–SYRIA, each tablet contains: 75 mg of CLP and (Exp. 04.2022).

(3) **Norgrel Plus,** F.C. Tablet, UNIPHARMA, Damascus–SYRIA, each tablet contains: 75 mg of CLP and 75 mg ASPIRIN (Exp. 09.2020).

(4) **Clopid,** F.C. Tablet, EL–SAAD**,** Aleppo–SYRIA, each tablet contains: 75 mg of CLP (Exp. 09.2022).

(5) **Plofexine,** F.C. Tablet, ASIA**,** Aleppo–SYRIA, each tablet contains: 75 mg of CLP (Exp. 05.2020).

(6) **Clotless,** F.C. Tablet, APHAMEA**,** Hama–SYRIA, each tablet contains: 75 mg of CLP (Exp. 01.2021).

Standard stock solutions

A stock standard solution of Clopidogrel Bisulphate

This solution was prepared by dissolving 42.63 mg from clopidogrel bisulphate in 100 mL methanol ($1x10^{-3}$ $mol.L^{-1}$), then dilute 1.000 mL and 0.100 mL from this solution to 100 mL ($1x10^{-5}$ and $1x10^{-6}$ $mol.L^{-1}$).

Working solutions

The stock solutions were further diluted to obtain working solutions daily just before use in the ranges of CLPB: 0.005, 0.008, 0.010, 0.020, 0.040, 0.060, 0.080, 0.100, 0.200, 0.300, 0.400, 0.600, 0.800, 1.000, 1.200, 1.25, 1.50, 1.75 and 2.00 µ$mol.L^{-1}$ (2.0990, 3.3584, 4.1980, 8.3960, 16.792, 25.188, 33.584, 41.980, 83.960, 125.94 167.92, 251.88, 335.84, 419.80, 503.76, 524.75, 629.70, 734.65 and 839.60 $ng.mL^{-1}$) by dilution of the volumes: 0.125, 0.200, 0.250 mL from stock standard solutions $1x10^{-6}$ $mol.L^{-1}$ and 0.050, 0.100, 0.150, 0.200, 0.250, 0.500, 0.750, 1.000, 1.500, 2.000, 2.500, 3.000, 3.125, 3.750, 4.375 and 5.000 mL from stock standard solutions $1x10^{-5}$ $mol.L^{-1}$ were transferred into 25 mL volumetric flask, diluted with 0.04 M Britton-Robinson buffer solution.

Supporting electrolyte

Britton Robinson, H_3PO_4-Na_2HPO_4, lithium perchlorate, sodium chloride, borax, sodium acetate (HAc-NaAc) buffers at pH (4.0-10.0) were used.

Procedure for pharmaceutical formulations

Contents of 20 tablets of each studied pharmaceutical formulations were weighted accurately, crushed to a fine powder and mixed well. Equivalent weight of contents of one tablet was solved in 50 mL methanol by using ultrasonic, filtered over a 100 mL flask and diluting to 100 mL with methanol, which content as the follows: 750 µg.mL^{-1} for all studied pharmaceutical formulations content 75 mg/tab.

These solutions were prepared daily by diluting 10 µL (0.010 mL) from stock solutions of pharmaceutical formulations into 100 mL volumetric flask, diluted with 0.04 M Britton-Robinson buffer solution to the mark (each solution contents 75 ng.mL^{-1} of CLP (0.2331x10^{-6} mol.L^{-1}).

Analytical procedure

Differential pulse voltammetry (DPV)

25 mL of working standard of Clopidogrel bisulphate (CLPB) or working solutions of pharmaceuticals was transferred to the cell. The solution was deoxygenated with N_2 gas for 500 s. The potential studied range was from -1050 to -1500 mV (versus Ag/AgCl) with differential pulse voltammetric analysis using hanging mercury drop electrode in the optimum conditions were applied. The peak height was measured at -1233 to -1246 mV in 0.04 M Britton-Robinson buffer solution at pH 6.5.

Differential pulse adsorptive stripping voltammetry (DPAdSV)

A 25 mL volume of working solution containing an appropriate concentration of CLPB was transferred into an electrochemical cell. The solution was deoxygenated with N_2 gas for 500 s. The accumulation potential (E_{acc}) -600 mV, accumulation time (t_{acc}) 80 and 160 s were applied. The potential scanned from -800 to -1500 mV (versus Ag/AgCl) using DPAdSV with HMDE in the optimum conditions were studied. The peak height (I_p) was measured at -1225 to -1325 mV in 0.04 M Britton-Robinson buffer solution at pH 6.5.

Results and Discussion

Voltammetric behavior of CLPB on HMDE

The reduction peak current (I_P) appears at potential -1233 to -1246 mV using DPV method. The values of Ip increases with increasing the concentration of CLPB from 6x10^{-8} – 2.0 x10^{-6} mol.L^{-1}(0.060-2.000 µM of CLPB), see Fig.1, curve 1.

In using DPAdSV method, I_p appears at potential -1225 to -1325 mV in 0.04 M Britton-Robinson buffer solution at pH 6.5 and I_p increases with increasing the concentration of CLPB from 4x10^{-8} –1.50x10^{-6} mol.L^{-1} (0.040-1.500 µM of CLPB) at t_{acc} 80 s, see Fig.1, curve 2 and 5x10^{-9} – 3.0x10^{-7} mol.L^{-1} (0.0050-0.300 µM of CLPB) at t_{acc} 160 s, see Fig.1, curve 3.

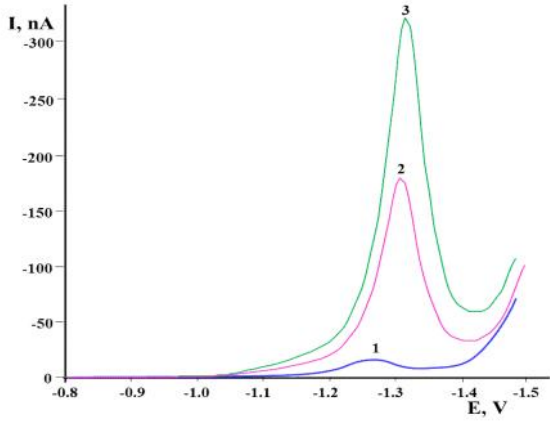


Fig.1: The polarograms for determination of CLPB in presence of 0.04 M Britton-Robinson buffer at pH 6.5: 1 – 1.0 µM CLPB using DPV (sweep rate 4 mV/s, t.step 1.0 s, U.step 4 mV); 2- 0.2 µM CLPB using DPAdSV at t_{acc} 80 s, 3- 0.2 µM CLPB using DPAdSV at t_{acc} 160 s. (Sweep rate 20 mV/s, t.step 0.3 s, U.step 6 mV, purge gas N_2, purge time 500 s, U.amplitude -100 mV, drop size 9, t.meas 30 ms, t.pulse 35 ms, temperature 25°± 5°C).

The effect of supporting electrolytes (buffer) and pH

The influence of pH from 5.0–10.0 using 0.04 M Britton-Robinson buffer on I_p and E_p was studied. The values of I_p (which is calculated starting from the current of the electrolyte) increase with increasing pH value of 5.5 to 6.25, then become semi-fixed until pH 7.50 and finally decrease until pH 10. While E_p values are growing a positive value from -1325 mV (when pH 5.5) to -1250 mV (when pH 7.5) then become semi-fixed until pH 10, see Figure 2. It was found that the best pH solution was 6.5 using DPV and DPAdSV.

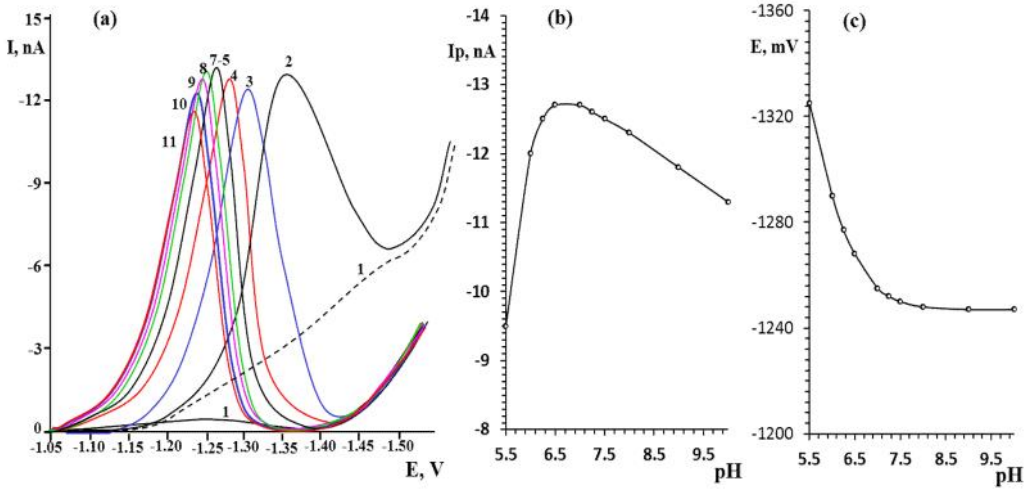


Fig.2: (a) The effect of pH solution on polarograms of CLPB (1.0 µM) using DPV and HMDE at pH: 1- electrolyte; 2- 5.50; 3- 6.00; 4- 6.25; 5- 6.50; 6- 7.00; 7- 7.25; 8- 7.50; 9- 8.00; 10- 9.00 and 11- 10.00 in 0.04 M Britton-Robinson buffer (Purge gas N_2, purge time 500 s, sweep rate 4 mV/s, U. amplitude -100 mV, drop size 9, t. meas 30 ms, t. pulse 35 ms, t. step 1.0 s, U. step 4 mV, temperature 25°± 5°C). (b) The effect of pH solution on I_p, (c) The effect of pH solution on E_p.

The electrochemical behavior of clopidogrel bisulphate was studied in various supporting electrolytes such as Britton Robinson, sodium chloride, lithium perchlorate, sodium acetate (HAc-NaAc), di-sodium hydrogen phosphate dodecahydrate, borax buffers were studied at pH (5.0-10.0). The best definition of the analytical signals was found in Britton-Robinson buffer at pH 6.5 and concentration 0.04 M. The effect of supporting electrolytes (buffer) on the I_p and E_p were studied. The values of E_p were -1268, -1270, -1295, -1330, -1320 and -1345 mV for the mentioned buffers, respectively, see Figure 3.

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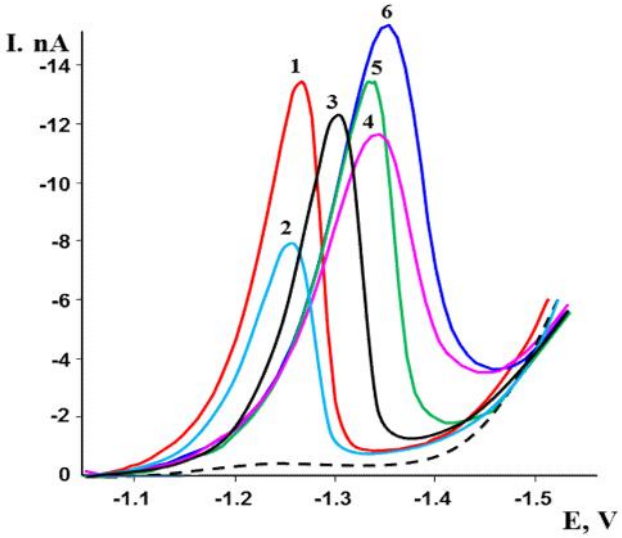

Fig.3: The effect of buffer solutions on polarograms of CLPB (1.00 µM) using DPV, HMDE in 0.04 M buffers and pH 6.5: 1- Britton-Robinson, 2- NaCl, 3- $LiClO_4.3H_2O$, 4- HAc-NaAc, 5- $Na_2HPO_4.12H_2O$, 6- $Na_2B_4O_7$ (Purge gas N_2, purge time 500 s, sweep rate 4 mV/s, U. amplitude -100 mV, t. meas 30 ms, t. pulse 35 ms, t. step 1.0 s, U. step 4 mV, drop size 9, temperature 25°± 5°C).

The effect of temperature and time

The effect of temperature and time on the electrochemical behavior of CLPB was studied at different values (15-35^{o}C and 5-60 min) by continuous monitoring of the I_p. It was found that, the value of I_p was not affected by temperature between 20 to 30^{o}C (the temperature at 25±5°C was used). The effect of waiting time was determined at laboratory ambient temperature (25±5°C). It was found that, the value of I_p was not affected by time between 5 to 60 min.

The effect of negative pulse amplitude (U.ampl)

The effect of negative pulse amplitude, U.ampl (Pulse amplitude of the voltage pulse superimposed on the direct voltage) between -10 to -100 mV on I_p and E_p by DPV and DPAdSV. I_p linearly increases with increasing amplitude value until -100 mV. While E_p stay semi-fixed. The value -100 mV was better than another's.

The effect of time pulse (t.pulse)

The effect of time pulse, t.pulse (Time interval during which a voltage pulse is superimposed on the direct voltage) between 35-100 ms on polarograms was as the follows: I_p decreases with increasing time pulse, the peak was more symmetrical and I_p was the highest when the t.pulse value of 35 ms by DPV and DPAdSV.

The effect of time interval for voltage step (t.step)

I_p linearly decreases with increasing t.step (Time interval after which the voltage in the sweep is increased or decreased by the value of U.step) between 0.1 - 2.5 s, the values of the preferred t.step were 1.0 s and 0.3 s for using DPV and DPAdSV, respectively.

The effect of measurement time (t.meas)

I_p increases with increasing t.meas (Time during which the current is measured. Measurement is performed at the end of the time interval t.step immediately before the pulse start and at the end of the pulse) between 2-32 ms, while E_p remains quasi-static. The value of the preferred t.meas was 30 ms using DPV and DPAdSV.

The effect of the accumulation potential (E_{acc})

The dependence of the differential pulse adsorptive stripping peak current on the accumulation potential (E_{acc}) +50 to -1200 mV at accumulation time (t_{acc}) 80 was examined. It was found that the maximum response for CLPB occurs with E_{acc} equal to -600 mV on HMDE electrode, see Fig.4. It was found that the change of t_{acc} does not affect the choice of the accumulation potential.

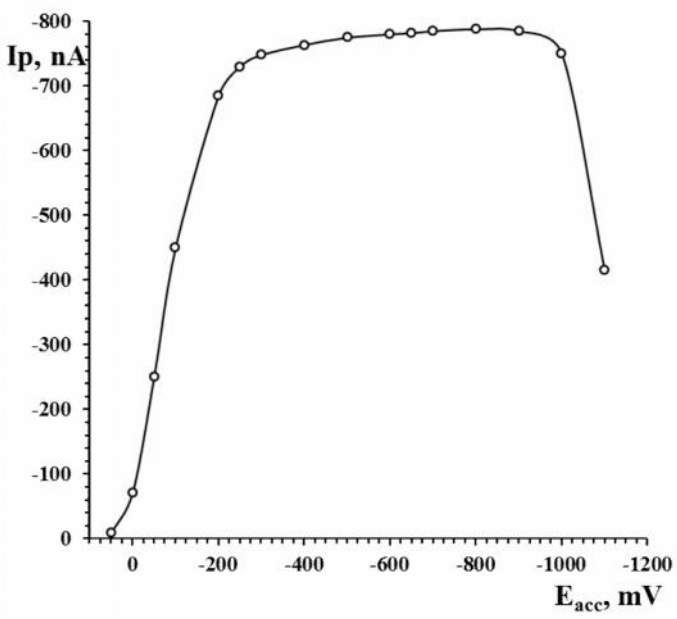

Fig.4: The effect of E_{acc} on I_p of CLPB (1.0 µM) using DPAdSV and HMDE at t_{acc} 80 in presence 0.04 M Britton-Robinson buffer (Purge gas N_2, purge time 500 s, sweep rate 20 mV/s, U. amplitude -100 mV, drop size 9, t. meas 30 ms, t. pulse 35 ms, t. step 0.3 s, U. step 6 mV, temperature 25°± 5°C).

Effect of accumulation time (t_{acc})

The peak current depended on the accumulation time (t_{acc}) for CLPB concentrations were studied at E_{acc} -600 mV. The peak current increases with increasing t_{acc}. The best t_{acc} was 80 s for CLPB concentrations $4x10^{-8}$-$1.5x10^{-6}$ M, while t_{acc} was160 s for CLPB concentrations $5x10^{-9}$ - $3x10^{-7}$ M on HMDE electrode at pH 6.5, see Fig.5. The optimum parameters for DPV and DPAdSV determination of CLPB were selected and presented in the (Table 1).

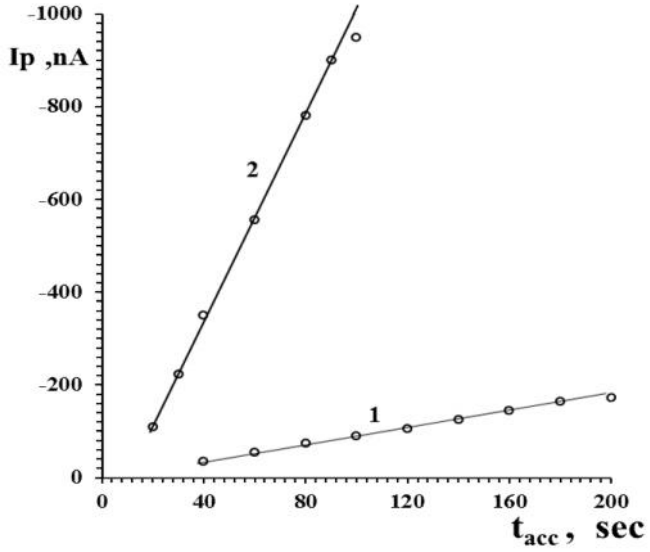

Fig.5: The effect of t_{acc} on I_p of CLPB using DPAdSV and HMDE at E_{acc} -600mV in presence 0.04 M Britton-Robinson buffer: 1- 0.1 µM of CLPB, 2- 1.0 µM CLPB (Purge gas N_2, purge time 500 s, sweep rate 20 mV/s, U. amplitude -100 mV, drop size 9, t. meas 30 ms, t. pulse 35 ms, t.

Table 1: The optimum parameters established for DPV and DPAdSV determination of CLPB.

Parameters	**Operating modes**	
	DPV	DPAdSV
Working electrode	Hanging mercury drop electrode (HMDE)	
Supporting electrolyte	0.04 M Britton-Robinson buffer	
Solvent clopidogrel	Double distilled deionized water	
Purge gas	Pure N_2 for 500 s	
Value of pulse amplitude	-100 mV	
t. pulse	35 ms	
Drop modified size	9 (0.60 mm^2)	
Temperature of solution	25°± 5°C	
t.meas	30 ms	
Rot. speed	2000 rpm	
pH	6.5	
t.step	1 s	0.3 s
u.step	4 mV	6 mV
Scan rate	4 mV/s	20 mV/s
Initial potential	-1050 mV	-800 mV
Final potential	-1500 mV	-1500 mV
Accumulation potential	-	-600 mV
Peak potential	-1233 to -1246 mV	-1225 to -1325 mV
Accumulation time	-	80 s and 160 s

Analytical results

The analytical curves, I_p =f (C_{CLPB}) for the determination of CLPB at pH 6.5 in presence of 0.04 M Britton-Robinson buffer on HMDE by DPV showed good linear $6x10^{-8}$-$2.0x10^{-6}$ mol.L^{-1} (25.188-839.60 ng.mL^{-1}), at pH 6.5 and by DPAdSV with E_{acc} -600 mV, t_{acc} 80 s or 160 s showed too good linear $4x10^{-8}$-$1.5x10^{-6}$ and $5x10^{-9}$-$3x10^{-7}$ mol.L^{-1} (16.790-629.70 and 2.0990-125.940 ng.mL^{-1}), see (Figures 6-8). Regression equations and correlation coefficient were as in Tables (2-4). This method showed very sensitive results for the determination of CLPB by DPAdSV more than that obtained using DPV.

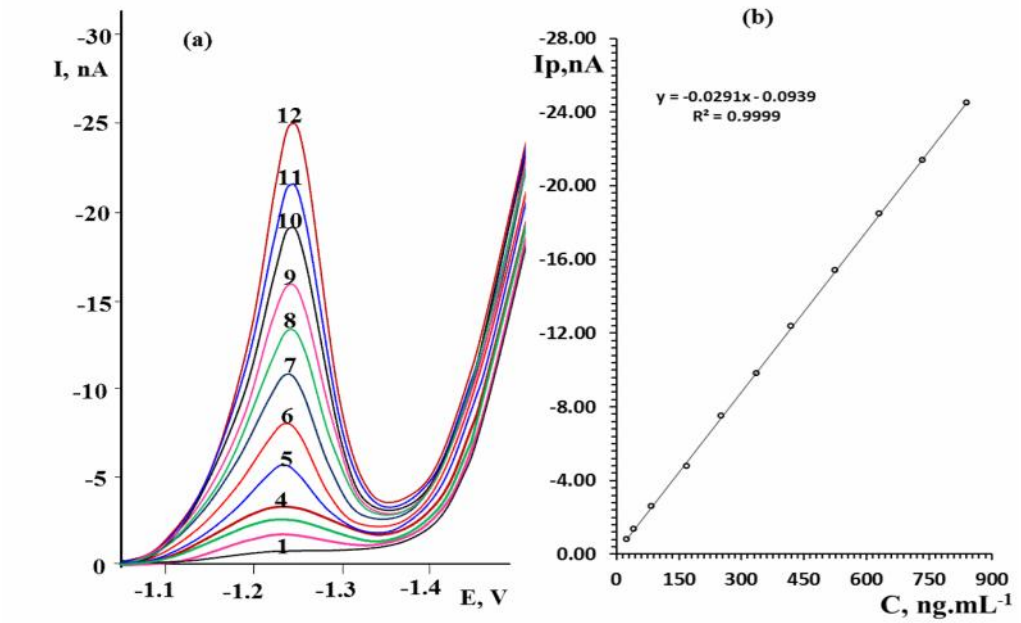

Fig.6: (a) The DPV Curves of CLPB in presence of 0.04 M Britton-Robinson buffer using HMDE at pH 6.5: 1- electrolyte, 2- 25.188 , 3- 41.980, 4- 83.980, 5- 167.92, 6- 251.88, 7- 335.840, 8- 419.800, 9- 524.75, 10- 629.700, 11- 734.650, and 12- 839.600 ng.mL^{-1}. (b) Calibration curves for determination of CLPB (Purge gas N_2, purge time 500 s, sweep rate 4 mV/s, U.amplitude -100 mV, drop size 9, t.step 1.0 s, t. meas 30 ms, t.pulse 35 ms, U.step 4 mV, temperature 25°± 5°C).

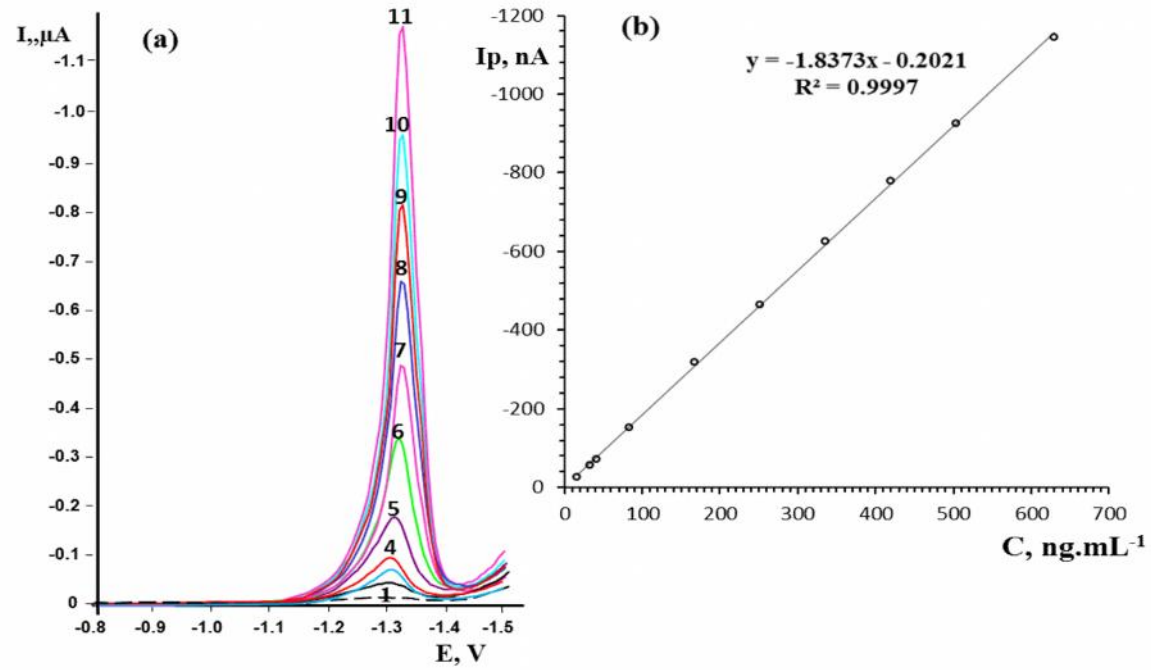

Fig.7: (a) The DPASdV Curves on HMDE of CLPB at E_{acc} -600 mV, t_{acc} 80 s in presence of 0.04 M Britton-Robinson buffer at pH 6.5: 1- electrolyte, 2- 16.792, 3- 33.584, 4- 41.980, 5- 83.960, 6- 167.920, 7- 251.880, 8- 335.840, 9- 419.800, 10- 503.760 and 11- 629.700 ng.mL^{-1}. (b) Calibration curves for the determination of CLPB (Purge gas N_2, purge time 500 s, sweep rate 20 mV/s, U.amplitude -100 mV, drop size 9, t.step 0.3 s, t. meas 30 ms, t.pulse 35 ms, U.step 6 mV, temperature 25°± 5°C).

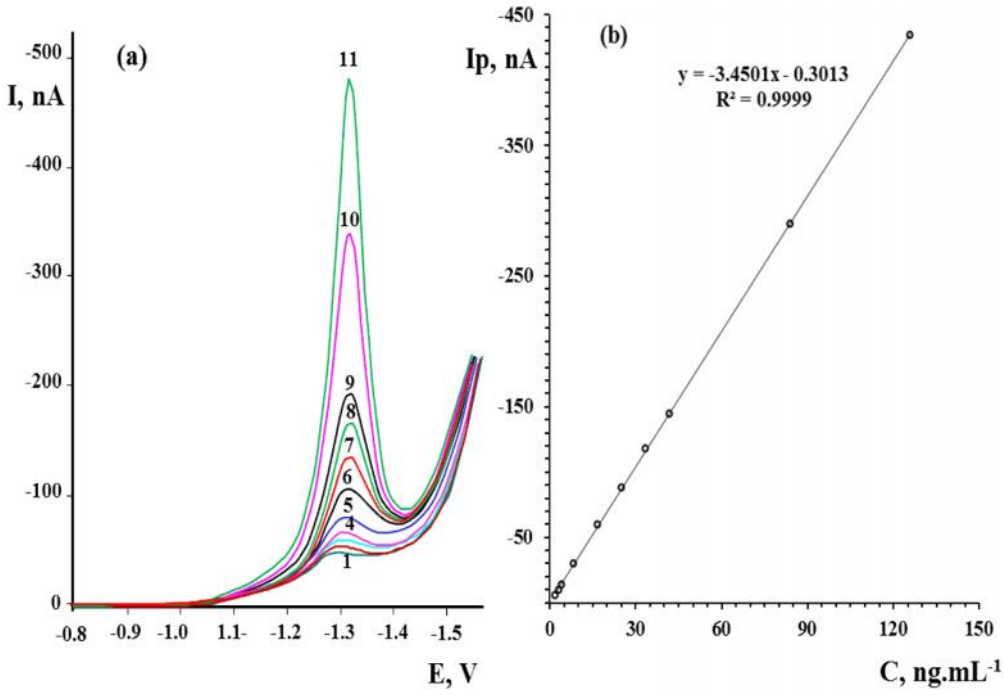

Fig.8: (a) The DPASdV Curves on of CLPB at E_{acc} -600 mV, t_{acc} 160 s in presence of 0.04 M Britton-Robinson buffer using HMDE at pH 6.5: 1- electrolyte, 2- 2.0990, 3- 3.3584, 4- 4.1980, 5- 8.3960, 6- 16.392, 7- 25.188, 8- 33.584, 9- 41.980, 10- 83.960 and 11- 125.940 ng.mL^{-1}. (b) Calibration curves for the determination of CLPB (Purge gas N_2, purge time 500 s, sweep rate 20 mV/s, U.amplitude -100 mV, drop size 9, t.step 0.3 s, t. meas 30 ms, t.pulse 35 ms, U.step 6 mV, temperature 25°± 5°C).

Table 2: Analytical parameters for determination of clopidogrel using DPV and DPAdSV methods.

Parameter	DPV	DPAdSV	
		t_{acc} , 80 s	t_{acc} ,160 s
Regression equations	y = -0.0291x-0.0939	y = -1.8373x-0.2021	y = -3.4501x-0.3013
Concentration range, ng.mL^{-1}	25.188-839.60	16.790-629.70	2.099-125.940
Concentration range, mol.L^{-1}	6×10^{-8} to 2.0×10^{-6}	4×10^{-8} to 1.5×10^{-6}	5×10^{-9} to 3×10^{-7}
R^2	0.9999	0.9997	0.9999
The lowest concentration, g.mL^{-1}	25.188	16.790	2.099
RSD%	2.8	2.6	3.2
LOD, ng.mL^{-1}	2.34	1.45	0.22
LOQ, ng.mL^{-1}	7.05	4.37	0.67

y: I_p, nA and x: C_{CLPB}, ng.mL^{-1}

Table 3: Determination of clopidogrel using DPV on HMDE with negative amplitude in presence of 0.04 M Britton-Robinson buffer at pH 6.5 (n=5, t=2.776).

Taken, x_i			Found, CLPB $\bar{x}$, ng.mL^{-1}	SD, ng.mL^{-1}	$\bar{x} \pm \frac{t.SD}{\sqrt{n}}$, ng.mL^{-1}	RSD%
«M	CLP ng.mL^{-1}	CLPB ng.mL^{-1}				
0.060	19.308	25.188	24.865	0.696	24.865 ± 0.864	2.8
0.100	32.180	41.980	41.821	1.046	41.821 ± 1.298	2.5
0.200	64.360	83.960	84.172	2.020	84.172 ± 2.508	2.4
0.400	128.720	167.920	165.970	3.817	165.970 ± 4.739	2.3
0.600	193.080	251.880	252.562	5.556	252.562 ± 6.898	2.2
0.800	257.440	335.840	334.780	7.030	334.780 ± 8.728	2.1
1.000	321.800	419.800	420.751	8.415	420.751± 10.447	2.0
1.250	402.250	524.750	525.130	8.927	525.130 ± 11.083	1.7
1.500	482.700	629.700	630.076	9.451	630.076 ± 11.734	1.5
1.750	563.150	734.650	733.343	10.270	733.343 ± 12.746	1.4
2.000	643.600	839.600	839.112	10.908	839.112 ±13.543	1.3

Table 4: Determination of clopidogrel using DPAdSV on HMDE with negative amplitude in presence of 0.04 M Britton-Robinson buffer at pH 6.5, t_{acc} 80, 160 s, E_{acc} -600 mV (n=5, t=2.776)

Accumulation time, s	Taken x_i			Found, CLPB $\bar{x}$, ng.mL^{-1}	SD, ng.mL^{-1}	$\bar{x} \pm \frac{t.SD}{\sqrt{n}}$, ng.mL^{-1}	RSD %
	«M	CLP ng.mL^{-1}	CLPB ng.mL^{-1}				
160	0.005	1.609	2.099	1.988	0.064	1.988 ± 0.079	3.2
	0.008	2.574	3.358	3.396	0.102	3.296 ± 0.126	3.0
	0.010	3.218	4.198	4.099	0.115	4.092 ± 0.143	2.8
	0.020	6.436	8.396	8.525	0.222	8.595 ± 0.275	2.6
	0.040	12.872	16.792	17.123	0.394	16.792 ± 0.489	2.3
	0.060	19.308	25.188	25.420	0.508	25.420 ± 0.631	2.0
	0.080	25.744	33.584	34.115	0.648	34.115 ± 0.805	1.9
	0.100	32.180	41.980	41.940	0.713	41.940 ± 0.885	1.7
	0.200	64.360	83.960	84.060	1.261	84.060 ± 1.565	1.5
	0.300	96.540	125.940	125.790	1.509	125.790 ± 1.874	1.2
80	0.040	12.872	16.792	15.985	0.416	15.985 ± 0.516	2.6
	0.080	25.744	33.584	32.667	0.784	32.667 ± 0.973	2.4
	0.100	32.180	41.980	41.586	0.915	41.586 ± 1.136	2.2
	0.200	64.360	83.960	83.886	1.678	83.960 ± 2.083	2.0
	0.400	128.720	167.920	172.760	3.110	172.760 ± 3.861	1.8
	0.600	193.080	251.880	252.980	4.048	252.980 ± 5.025	1.6
	0.800	257.440	335.840	339.870	4.758	339.870 ± 5.907	1.4
	1.000	321.800	419.800	423.560	5.506	423.560 ± 6.836	1.3
	1.200	386.160	503.760	503.460	6.042	503.460 ± 7.500	1.2
	1.500	482.700	629.700	624.190	6.242	624.190 ± 7.749	1.0

The proposed mechanism of clopidogrel on HMDE

On the basis of the experimental results obtained DPV and DPAdSV at pH 6.5, the mechanism could be suggested for the voltammetric reduction of clopidogrel, which corresponds to the usual reduction mechanism for the >C=O group. The electrochemical reaction is suggested to proceed as follows:

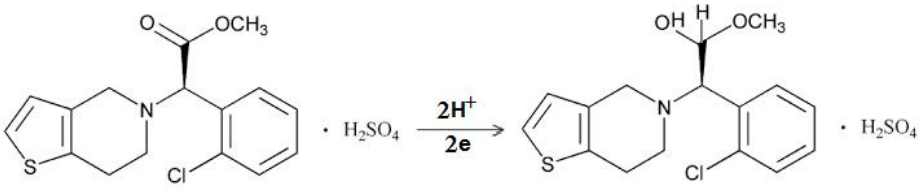

Figure 9: Electrochemical mechanisms of clopidogrel.

Applications

Many applications for the determination of clopidogrel in some Syrian pharmaceutical preparations (in presence a same amount aspirin) using differential pulse polarographic analysis on hanging mercury drop electrode with negative amplitude using DPV and DPAdSV in presence of 0.04 M Britton-Robinson buffer at pH 6.5 according to the optimal conditions were proposed. The amount (m) of CLPB in one tablet was calculated from the following relationship:

m=h. m', where: m' is the amount of CLPB in tablet calculated according to the regression equation of calibration curve, h conversion factors are equal to 1 for all pharmaceuticals content 75 mg/tab of CLP. The results of quantitative analysis for CLPB in pharmaceutical preparations were summarized in Tables 5. The proposed method was simple, direct and successfully applied to the determination of CLPB in pharmaceuticals without any interference from excipients. Average assay ranged between 99.4 to 102.0%. Therefore, the presented method can be recommended for routine analysis of CLP (as CLPB) in pharmaceutical formulations.

Table 5: Determination of CLP in some Syrian pharmaceutical preparations using DPV and DPAdSV (at E_{acc} -600 mV, t_{acc} 80 s and pH 6.5) on HMDE in presence of 0.04 M Britton-Robinson buffer at according to the optimal conditions (n=5, t=2.776)

Commercial name	Method	Label Claim of CLP, mg/cap.	Mean ±SD (as CLP), mg/ cap.	RSD%	Assay %
***Pharma GreL*, F.C.Tablet PHARMASYR**	DPV	75	74.6	2.1	99.4
	DPAdSV		74.8	2.0	99.7
***Plaraz*, F.C. Tablet AL–RAZI**	DPV	75	76.2	2.1	101.6
	DPAdSV		76.5	1.9	102.0
***Norgrel Plus*, F.C. Tablet, UNIPHARMA**	DPV	75	75.6	2.2	100.8
	DPAdSV		75.4	1.8	100.5
***Clopid*, F.C. Tablet, EL–SAAD**	DPV	75	74.5	2.2	99.3
	DPAdSV		74.9	2.0	99.9
***Plofexine*, F.C. Tablet, ASIA**	DPV	75	76.4	2.1	102.0
	DPAdSV		76.6	1.8	101.9
***Clotless*, F.C. Tablet, APHAMEA**	DPV	75	76.3	2.0	101.7
	DPAdSV		76.5	1.7	102.0

Method validation

The developed method for simultaneous estimation of CLPB has been validated in accordance with the International Conference on Harmonization guidelines (ICH) [28].

Selectivity

Several other components were examined under the conditions that had been optimized for clopidogrel determination. The results show that aspirin did not interfere when it presents at same amount with clopidogrel using DPV and DPAdSV at pH 6.5.

Linearity

In the proposed methods, linear plots (n=5) with good correlation coefficients were obtained in the concentration ranges of y = - 0.0291x - 0.0939 (R^2=0.9999) by DPV for the concentration from 25.188-839.60ng.mL^{-1}, y =-1.8373 x - 0.2021 (R^2=0.9997) and y = -3.4501 x - 0.3013 (R^2=0.9999) on HMDE electrode by DPAdSV for the concentration from 16.790-629.70 ng.mL^{-1} and 2.0990-125.940 ng.mL^{-1} at t_{acc} 80 s and t_{acc} 160 s respectively. In this method a very low concentration 25.188 ng.mL^{-1} (6.0×10^{-8} mol.L^{-1}), 16.790 ng.mL^{-1} (4.0×10^{-8} mol.L^{-1}) and 2.099 ng.mL^{-1} (5.0×10^{-9} mol.L^{-1}) of CLPB, respectively.

Precision and Accuracy

The precision and accuracy of proposed method were checked by recovery study by addition of standard drug solution to pre-analyzed sample solution at three different concentration levels (80%,100% and 120%) within the range of linearity for CLPB. The basic concentration level of sample solution selected for spiking of the CLPB standard solution was 41.980 ng.mL^{-1}. The proposed method was validated statistically and through recovery studies, and was successfully applied for the determination of CLPB in pure and dosage forms, Table 6.

Table 6 : Results of recovery studies (n=5, t=2.776).

Level	Recovery %		
	DPV	DPAdSV t_{acc} 80 s	DPAdSV t_{acc} 160 s
80%	100.8	100.0	99.9
100%	99.7	100.4	101.3
120%	100.4	101.0	101.1

Repeatability

The repeatability was evaluated by performing 10 repeat measurements for 41.980 ng.mL^{-1} of CLPB using the studied methods under the optimum conditions. The found amounts of CLPB ($\bar{x}$ ±SD) were 41.720±0.924 ng.mL^{-1}, 41.854±0.710 ng.mL^{-1} and 41.572±0.611 ng.mL^{-1}, the percentage recovery were found to be 99.38±2.2 (with RSD of 0.022), 99.7±1.7 (with RSD of 0.017) and 99.0±1.5 (with RSD of 0.015) using DPV and DPAdSV at t_{acc} 80 s and 160 s, respectively. These values indicate that the proposed method has high repeatability for CLPB analysis.

Sensitivity limit of detection [LOD] and limit of quantitation [LOQ]

The sensitivity of the presented method was evaluated by determining the LOD and LOQ. The values of LOD for CLPB were 2.34, 1.45 and 0.22 ng.mL^{-1}, and LOQ were 7.05, 4.37 and 0.67 ng.mL^{-1} at pH 6.5 and E_{acc} -600 mV using DPV and DPAdSV (t_{acc} 80 s and 160 s), respectively.

Robustness

The robustness of the method adopted is demonstrated by the constancy of the current peak (I_p) with the deliberated minor change in the experimental parameters such as the change in the concentration of excipients, temperature (25±5^{o}C), pH (6.5±0.20), and accumulation potential -600 ±5 mV). This table indicates that the robustness of the proposed methods was good (I_p was measured and assay was calculated for five times), Table 7.

Table 7: Robustness of the proposed DPV and DPAdSV methods at HMDE for determination of CLPB (n=5, t=2.776).

Parameters	Experimental parameter variation	Average recovery (%) C_{CLPB} = 41.98 ng.mL^{-1}		
		DPV	DPAdSV	
			t_{acc} 80 s	t_{acc} 160 s
Temperature	20°C	99.7	99.8	99.9
	30°C	100.6	100.7	101.0
pH	6.3	99.6	99.9	100.1
	6.7	100.3	100.6	100.3
Accumulation potential	-595 mV	-	100.2	99.8
	-605 mV	-	100.7	101.3

Specificity

The specificity of the method was ascertained by analyzing standard CLPB in presence of excipients. These findings prove that the suggested methods are specific for determination of the investigated drugs without interference from the co-formulated adjuvants.

Conclusion

Electroreduction and adsorption of clopidogrel (CLP) as bisulphate (CLPB) in pure and pharmaceutical dosage forms using DPV and DPAdSV at HMDE has been studied. E_p of CLPB using DPV was between the range -1233 to -1246 mV (versus Ag/AgCl), at pH 6.5. Linear calibration graph were in the concentration ranges of 0.060-2.000µmol.L^{-1} (25.188-839.600 ng.mL^{-1}) with relative standard deviations did not exceed 2.8%. DPAdSV was applied for determination of CLPB at pH 6.5. E_p was between -1225 to -1325 mV (versus Ag/AgCl). Linear calibration graphs at E_{acc}= -600 mV, t_{acc}= 80 s and 160 s, were of 0.060-1.500 µmol.L^{-1} (16.792-629.700 ng.mL^{-1}) and 0.0050-0.300 µmol.L^{-1} (2.099-125.940 ng.mL^{-1}) with relative standard deviations did not exceed 2.6% and 3.2%, respectively. It was found that the use of DPAdSV have increased sensitivity 1.5 and 12 times at t_{acc} 80 s and 160 s, respectively. These methods give good results for the determination of CLPB in pure and different dosage forms.

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