INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES

(p-ISSN: 2348-5213: e-ISSN: 2348-5221)

www.ijcrcps.com

DOI: 10.22192/ijcrcps Coden: IJCROO(USA) Volume 7, Issue 5 - 2020

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2020.07.05.001

Behavior and Quantification of Clopidogrel Bisulphate in Pure and Pharmaceutical Dosage Forms Using Differential Pulse Polarography With Static Mercury Drop Electrode

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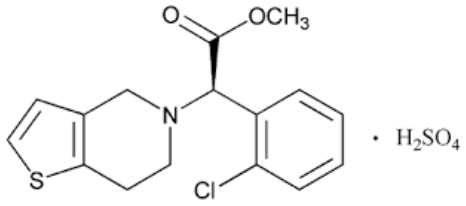
Abstract

Differential pulse polarographic analysis (DPPA) was applied by using static mercury drop electrode (SMDE) for quantification of clopidogrel bisulphate (CLPB) in pure and pharmaceutical dosage forms. The optimum conditions for the polarographic signal were determined and a study was made of the different parameters affecting the electrochemical process. The best definition of the analytical signals was found in di-sodium hydrogen phosphate buffer (0.02 M) at pH 8.5. Under the optimum conditions, liner calibration graph, $I_p=f(C_{CLP})$ was obtained in the concentration ranges of 0.25 µM (0.08055 µg.mL^{-1}) to 30 µM (9.6546 µg.mL^{-1}) at -1278 to -1312 mV (versus Ag/AgCl) with relative standard deviations (RSD) did not exceed 2.2% for the concentrations of CLP (0.0805µg.mL^{-1}). Regression analysis showed a good correlation coefficient (R^2=0.9999) between I_p and concentration over the mentioned range. The limit of detection (LOD) and the limit of quantification (LOQ) were to be 0.010 and 0.030 µg.mL^{-1}, respectively. The proposed method was validated for linearity, precision and accuracy, repeatability, sensitivity (LOD and LOQ), robustness and specificity. The developed method is applicable for the determination of CLP in pure and different dosage forms in presence a same amount of aspirin (ASP) with average recovery of 98.9 to 101.8% and the results are in good agreement with those obtained by the HPLC reference method.

Keywords: Differential pulse polarography; Static mercury drop electrode, Clopidogrel.

Introduction

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 g/mol of CLPB (draw CLP is 321.8 g/mol) [1,2].



Scheme 1 Chemical structure of clopidogrel bisulphate (CLPB)

Clopidogrel is as a potent anti-platelet aggregation agent, has become available in the market. The medical properties, pharmaco dynamics, pharmacokinetics and various aspects of this compound, have been published [3-5].

The literatures for the quantification of clopidogrel were including potentiometric method [6-8] and voltammetry [9-15], spectrophotometry [16-18], and high performance liquid chromatography [19-20].

The construction and general performance characteristics of membrane potentiometric sensors responsive to the clopidogrel bisulphate drug are described. The sensors are based on the use of ion-association complexes of clopidogrel bisulphate (CLPB) with tungstosilicic acid haydrate, as exchange sites in a PVC matrix (Coated wire) (I), PVC silicon oxide nanoparticles (II) and graphine -nanographine oxide (III), The sensors show a fast, stable and near- Nernstian response for the mono and di charge cation and di charge anion of CLP over the concentration range1×10^{-5} - 10^{-2} M for (I), 1×10^{-7} - 10^{-3} M for both (II) and (III) at 25 °C over the pH range 1.4 - 3.7, 1.4 - 4.7 and 1.4 – 10.2 for CLP (I , II and III) sensors with slope of 54.39 ± 0.4 ,30.19 ± 0.4 and 28.1 ± 0.2 respectively. The new sensors were used for the determination of CLP in tablets, Urine and Serum compared with standard method using UV and potentiometric titration [8].

A novel modified glassy carbon electrode based on poly aspartic acid-Fe_3O_4 nanoparticle/multi-walled carbon nanotubes composite (PAA-FeNPs-MWCNTs/GCE) was developed and used as an efficient sensor for simultaneous determination of piroxicam (PRX) and clopidogrel (CLP) in the presence of uric acid (UA). The differential pulse voltammetry (DPV) results showed that under the optimal experimental conditions the obtained anodic peak currents were linearly proportional to concentration in the range of 0.2 - 80 μM with a detection limit of 76 nM for CLP [13].

The DNA binding properties of clopidogrel bisulphate, was examined by cyclic voltammetry, fluorescence and UV spectroscopy. The binding constant is calculated using absorbance and is found to be closer to that estimated in voltammetric measurements [14].

The effect of variation of concentration of aspirin on electrooxidation of clopidogrel bisulphate is studied by variation of scan rate and pH. With increase in sweep rate, the anodic peak current increases linearly with correlation coefficient 0.9819 indicating the electrooxidation process to be diffusion controlled. Spectrophotometrically, absorption maximum of aspirin was found to be 223nm and 275nm while that of clopidogrel bisulphate was found to be 227nm. In vitro interactions were carried out in simulated intestinal juice and gastric juice with pH 1 and 9 respectively.

It was found that absorption maximum of aspirin remained at 223nm while that of clopidogrel shifted to 203nm, confirming the interaction [15].

The static mercury drop electrode (SMDE) used successfully in polarographic analysis. The SMDE combines the features of the DME and HMDE: As with the DME, the drops are constantly renewed, but during the measurement the drop area is constant as in the HMDE case. In a subsequent voltage (U) sweep, the Hg drops are knocked off by the tapping mechanism after the time t.step set in the measurement mode. The SMDE is primarily used for sensitive measurements in which the surface of the mercury drop must be renewed for every measurement, but sensitive measurements decrease when get adsorption reaction on electrode. Further, less mercury is needed. On the other hand, the MME capillary is subjected to greater mechanical stress than in the SMDE case [21].

In the present research, differential pulse polarographic behavior and quantification of the clopidogrel in pure and pharmaceutical dosage forms using a static mercury drop electrode was applied. The method is easy, fast and sensitive for the determination of this compound in pure and in pharmaceuticals.

Materials and Methods

Equipment and Materials

A Metrohm 746 VA processor, A Metrohm 747 VA stand with a static mercury drop electrode (SMDE) 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 20 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.

We used working reference standard of clopidogrel (98.5%) was supplied by D.K. Pharma. Chem. Pvt. Ltd INDIA, (Mfg.11-2018, 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.

A commercial formulations (as tablets) were used for the analysis of CLP by using DPPA with SMDE 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 ($1x10^{-4}$ $mol.L^{-1}$)

This solution was prepared by dissolving 42.63 mg from clopidogrel bisulphate in 100 mL methanol ($1x10^{-3}$ $mol.L^{-1}$), then dilute 10.000 mL from this solution to 100 mL ($1x10^{-4}$ $mol.L^{-1}$).

Supporting electrolyte

Britton robinson, H_3PO_4-Na_2HPO_4, lithium perchlorate, sodium chloride, borax, sodium acetate (HAc-NaAc) buffer 0.02 $mol.L^{-1}$ at pH (6.0-10.0) were used.

Recommended Procedure

The stock solutions were further diluted to obtain working solutions daily just before use in the ranges of clopidogrel: 0.1050, 0.2100, 0.4199, 0.8398, 1.6796, 3.3592, 5.0388, 6.7184, 8.3980, 10.4975 and 12.5970 $\mu g.mL^{-1}$ of CLPB (equivalent 0.0805 0.1609, 0.3218, 0.6436, 1.2873, 2.5746, 3.8618, 5.1491, 6.4363, 8.0455 and 9.6546 of CLP $\mu g.mL^{-1}$) or 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, 12.00, 16.00, 20.00, 25.00 and 30.00 $\mu mol.L^{-1}$ by using of the volumes: 0.0625, 0.125, 0.250, 0.500, 1.000, 2.000, 3.000, 4.000, 5.000, 6.250, and 7.500 mL from stock standard solutions ($1x10^{-4}$ $mol.L^{-1}$) were transferred into 25 mL volumetric flask. 5.0 mL of supporting electrolyte was added, and diluted with double distilled water to the mark.

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 $\mu g.mL^{-1}$ for all studied pharmaceutical formulations content 75 mg/tab.

These solutions were prepared daily by diluting 100 µL (0.100 mL) from stock solutions of pharmaceutical formulations into 25 mL volumetric flask, diluted with H_3PO_4-Na_2HPO_4 buffer 0.02 M (pH 8.5) to the mark (each solution contents 3.000 $\mu g.mL^{-1}$ of CLP ($9.323x10^{-6}$ M).

Analytical procedure

25 mL of working standard of clopidogrel or working solutions of pharmaceuticals was transferred to the cell. The solution was deoxygenated with N_2 gas for 500 s. The potential range studied was from –1050 to –1500 mV versus Ag/AgCl with differential pulse polarographic analysis using static mercury drop electrode in the optimum conditions were applied.

Results and Discussion

Differential pulse polarographic behavior

The polarograms for concentration 0.25-30.0 $\mu mol.L^{-1}$ (0.105-12.5970 $\mu g.mL^{-1}$ of CLPB or 0.0805-9.6546 of CLP $\mu g.mL^{-1}$) in the optimal conditions (supporting electrolytes, pH, scan rate, initial potential, final potential, etc.) using DPPA at SMDE were studied. The best definition of the analytical signals was found in H_3PO4-Na_2HPO4 (0.02 M) buffer (pH 8.5) at -1278 to -1312 mV (versus Ag/AgCl).

The effect of supporting electrolytes (buffer)

The electrochemical behavior of clopidogrel was studied in various supporting electrolytes such as (Britton Robinson, di-sodium hydrogen phosphate dodecahydrate, sodium chloride, sodium acetate (HAc-NaAc), lithium perchlorate, borax buffers were studied at pH (6.0-10.0). The best definition of the analytical signals was found in di-sodium hydrogen phosphate dodecahydrate buffer (pH 8.5) at concentration 0.02 M. The effect of supporting electrolytes (buffer) on the I_p and E_p was studied. The values of E_p were -1247, -1279, -1283, -1284, -1305 and -1343 mV for the mention buffers, respectively, see Figure 1.

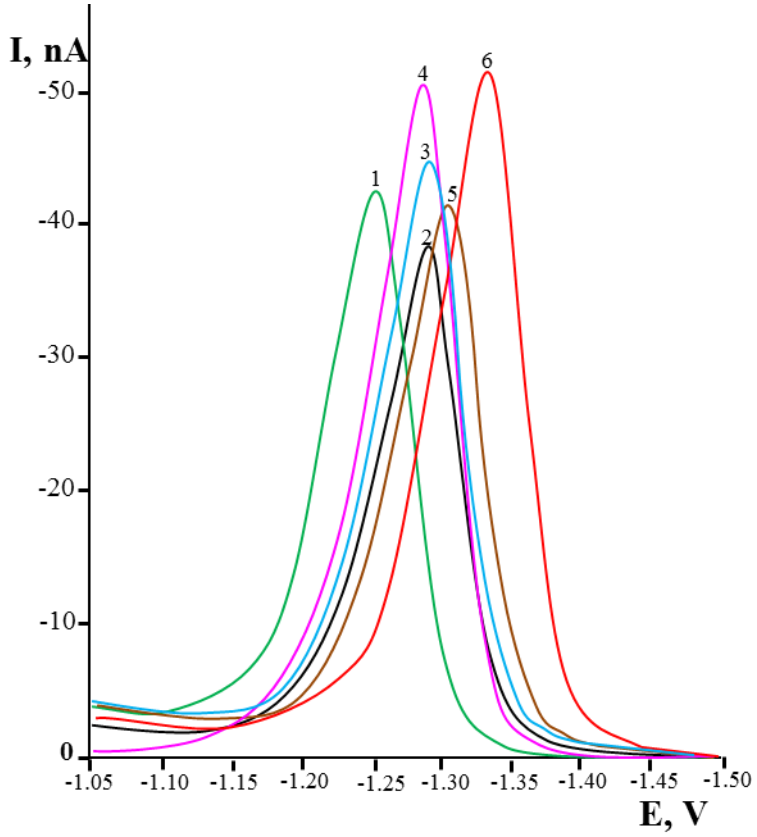


Fig.1: The effect of buffer solutions on polarograms of CLP (12 µM) using DPPA at SMDE buffers (0.02 M) at pH 8.5: 1- Britton-Robinson, 2- HAc-NaAc, 3- NaCl , 4- $Na_2HPO_4.12H_2O$, 5- $LiClO_4.3H_2O$, 6- $Na_2B_4O_7$ (Purge gas N_2, purge time 500s, sweep rate 5 mV/s, U. amplitude -100 mV, t. meas 30 ms, t. pulse 35 ms, t. step 1.6 s, U. step 8 mV, drop size 9, temperature 25°± 5°C).

The effect of pH

The influence of pH from 6.0 to 10.0 using di-sodium hydrogen phosphate dodecahydrate (0.02 M) buffer on I_p and E_p was studied. The values of I_p increase with increasing pH value of 6.0 to 8.0, then become semi-fixed until pH 9.0 and finally decrease until pH 10. While E_p values are growing a positive value from -1363 mV (when pH 6.0) to -1284 mV (when pH 9.0) then become semi-fixed until pH 10, see Figures 2,3.

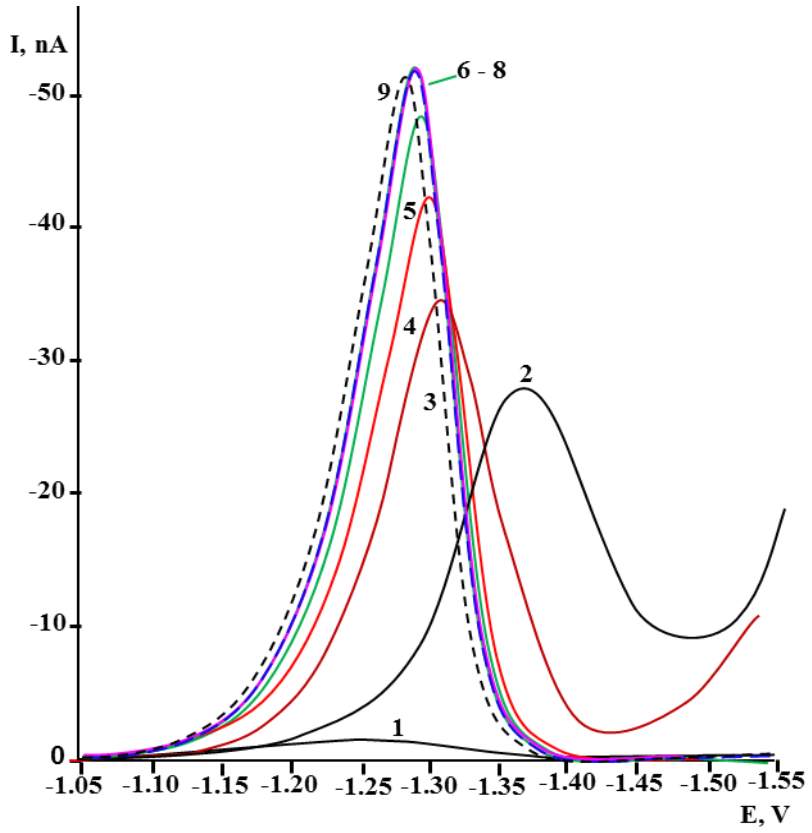


Fig.2: The effect of pH solution on polarograms of CLP (12 µM) using DPPA at SMDE at pH: 1- electrolyte; 2- 6.00; 3- 6.75; 4- 7.00; 5- 7.50; 6- 8.00; 7- 8.50; 8- 9.00; and 9- 10.00 ($Na_2HPO_4.12H_2O$ (0.02 M), Purge gas N_2, purge time 500 s, sweep rate 5 mV/s, U. amplitude -100 mV, drop size 9, t. meas 30 ms, t. pulse 35 ms, t. step 1.6 s, U. step 8 mV, temperature 25°± 5°C).

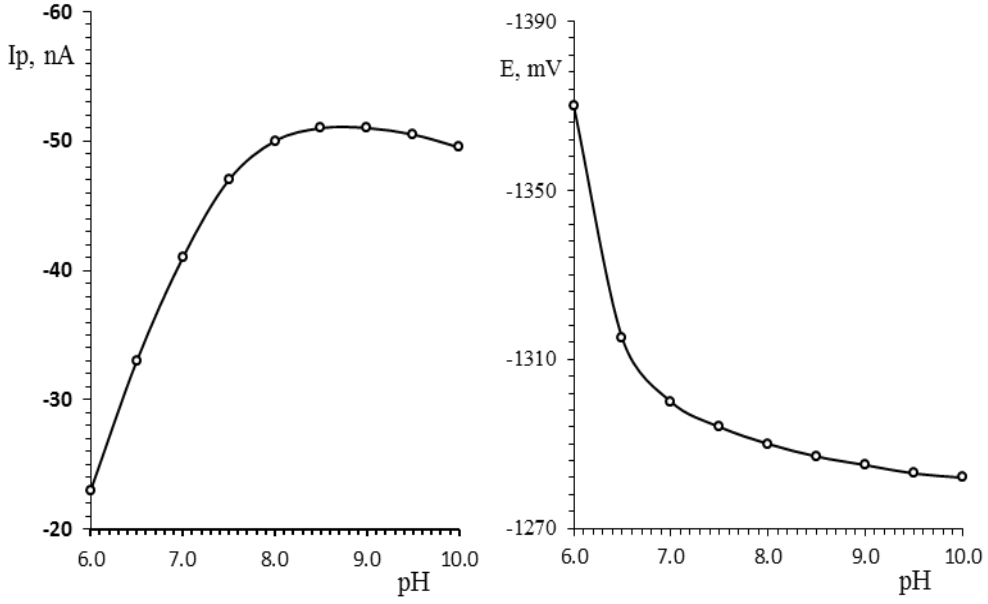


Fig.3: The effect of pH solution on E_p and I_p of CLP (12 µM) using DPPA at SMDE containing buffer $Na_2HPO_4.12H_2O$ (0.02 M) (Purge gas N_2, purge time 500s, sweep rate 5 mV/s, U. amplitude -100 mV, drop size 9, t. meas 30 ms, t. pulse 35 ms, t. step 1,6 s, U. step 8 mV, temperature 25°± 5°C).

The effect of negative pulse amplitude (U.ampl)

The effect of negative pulse amplitude (U.ampl) between -10 to -100 mV on I_p and E_p, 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 and Ip was the highest.

The effect of initial and final potential

The effect of initial and final potential on the I_p and E_p was studied. It was found that better initial potential was -1050 mV and better final potential was -1500 mV.

The effect of temperature and time

The effect of temperature and time on the electrochemical behavior of CLP 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 time pulse (t.pulse)

The effect of time pulse (35, 40, 50, 60, 70, 80, 90 and 100 ms) on polarograms was as the follows:

Ip decreases with increasing time pulse and Ep has become increasingly negative value (-1282 to -1265 mV) with increasing t.pulse. The peak was more symmetrical and Ip was the highest when the t.pulse value was 35 ms.

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

Ip linearly increases with increasing t.step (0.6, 0.8, 1.2, 1.6 and 2.0 s), while Ep remains fixed with increasing t.step. The value of the preferred t.step was 1.6 s.

The effect of measurement time (t.meas)

I_p increases with increasing t.meas. (4, 8, 12, 16, 20, 24, 28, 30, and 32 ms), while E_p remains quasi-static. The value of the preferred t.meas. was 30 ms.

The effect of drop size

I_p increases with increasing drop size from 1 to 9 size, While E_p stay semi-fixed with increasing drop size. The value of the preferred drop size was 9. The optimum parameters established for determination of CLP using DPPA on SMDE showed in Table 1.

Table 1: The optimum parameters established for determination of CLP using DPPA on SMDE.

Parameters	Operating modes
Working electrode	Static Mercury Drop Electrode (SMDE)
Supporting electrolytes (buffer)	di-sodium hydrogen phosphate 0.02 M
pH	8.5
solvent of clopidogrel	Methanol
Purge gas	Pure N_2
Purge time	500 s
Initial potential	-1050 mV
Final potential	-1500 mV
Scan rate	8 mV/s
t. meas	30 ms
Value of pulse amplitude	-100 mV
t. pulse	35 ms
t. step	1.6 s
Drop size	9
Temperature of solution	25°± 5°C

Calibration curves

Calibration curves for the determination of CLP using differential pulse polarographic analysis on SMDE with negative amplitude in di-sodium hydrogen phosphate (0.02 M) buffer at pH 8.5 were applied. One peak was observed in the range -1278 to -1312 mV (E_p). The peak current (I_p) was proportional to the concentration of CLPB over the ranges 0.1050-12.5970 $\mu g.mL^{-1}$ or 0.0805-9.6546 of CLP $\mu g.mL^{-1}$ (0.250-30.000 $\mu mol.L^{-1}$). The polarograms in the optimum conditions using DPPA at SMDE of CLP at different concentrations show in Figure 4. The regression equation were as the follows: y=-12.662x-0.1215, R^2=0.9999; y: I_p, nA and x: C_{CLP}, $\mu g.mL^{-1}$ see Figure 4.

Analytical results

Determination of CLP using DPPA on SMDE in the optimum conditions using analytical curves, $I_p=f(C_{CLP})$, showed that the accuracy was ready over the ranges of CLP concentration between 0.0805-9.6546 of CLP $\mu g.mL^{-1}$. The relative standard deviation (RSD) did not more than 2.2%, see Table 2. Limit of detection (LOD) and limit of quantitation (LOQ) for the determination of CLP by this method were as the follows: 0.010 and 0.030 $\mu g.mL^{-1}$, respectively.

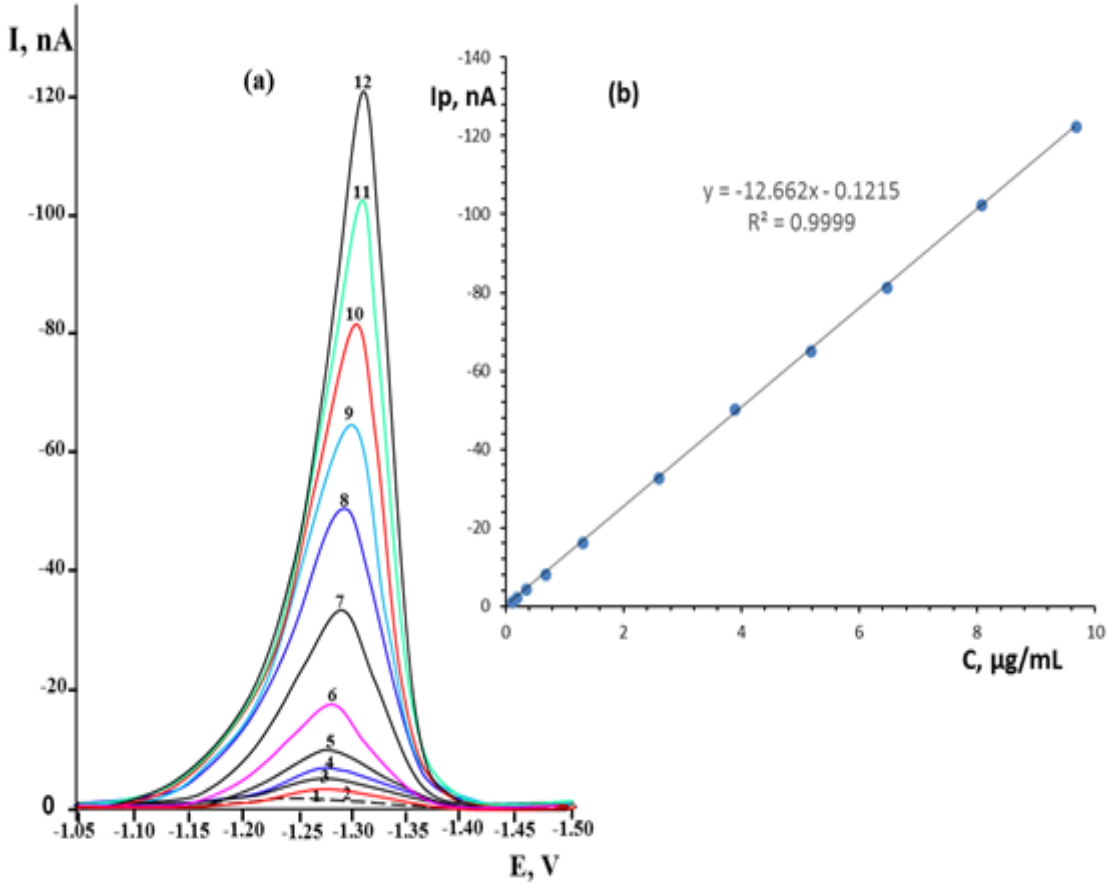


Fig.4: (a) The polarograms in the optimum conditions using DPPA on SMDE of CLP using DPPA at SMDE in $Na_2HPO_4.12H_2O$ 0.02 M buffer at concentrations: 1- electrolyte, 2- 0.0805, 3- 0.1609, 4- 0.3218, 5- 0.6436, 6- 1.2873, 7- 2.5746, 8- 3.8618, 9- 5.1491, 10- 6.4364, 11- 8.0455, 12- 9.6546 $\mu g.mL^{-1}$, (b) Calibration curves for the determination of CLP (purge gas N2, purge time 500 s, sweep rate 5 mV/s, U. amplitude -100 mV, t. meas 30 ms, t. pulse 35 ms, t. step 1.6 s, U. step 8 mV, temperature 25°± 5°C).

Table 2: Determination of clopidogrel using differential pulse polarographic analysis on SMDE with negative amplitude in di-sodium hydrogen phosphate (0.02 M) buffer at pH 8.5.

Taken x_i		Found *$\bar{x}$, $\mu g.mL^{-1}$	SD, $\mu g.mL^{-1}$	$\frac{SD}{\sqrt{n}}$, $\mu g.mL^{-1}$	$\bar{x} \pm \frac{t.SD}{\sqrt{n}}$, $\mu g.mL^{-1}$	RSD%
µM	$\mu g.mL^{-1}$ (CLP)					
0.25	0.0805	0.0817	0.0018	0.00805	0.0817± 0.00223	2.2
0.50	0.1609	0.1634	0.0033	0.00146	0.1634± 0.00406	2.0
1.00	0.3218	0.3282	0.0066	0.00294	0.3282± 0.00815	2.0
2.00	0.6436	0.6295	0.0120	0.00535	0.6295± 0.01485	1.9
4.00	1.2873	1.2787	0.0230	0.01029	1.2787± 0.02858	1.8
8.00	2.5746	2.5735	0.0438	0.01957	2.5735± 0.05432	1.7
12.00	3.8618	3.9628	0.0634	0.02836	3.9628± 0.07872	1.6
16.00	5.1491	5.1499	0.0824	0.03685	5.1499± 0.10230	1.6
20.00	6.4364	6.4246	0.0964	0.04310	6.4246± 0.11964	1.5
25.00	8.0455	8.0781	0.113	0.05058	8.0781± 0.14041	1.4
30.00	9.6546	9.6608	0.145	0.06481	9.6608± 0.1800	1.5

* n=5 t=2.776.

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 static mercury drop electrode with negative amplitude in di-sodium hydrogen phosphate 0.02 M buffer pH 8.5 according to the optimal conditions were proposed. The amount (m) of CLP in one tablet was calculated from the following relationship: m=h. m', where: m' is the amount of CLP in tablet calculated according to the regression equation of calibration curve, h conversion factors are equal to 25 for all pharmaceuticals content 75 mg/tab. The results of quantitative analysis for CLP in pharmaceutical preparations were summarized in Table 3. The proposed method was simple, direct and successfully applied to the determination of CLP in pharmaceuticals without any interference from excipients. Average assay ranged between 98.9 to 101.8%. The results obtained by this method agree well with the contents stated on the labels and were validated by RP-HPLC method [19]. Therefore, the presented method can be recommended for routine analysis of CLP in pharmaceutical formulations.

Table 3: Determination of CLP in some Syrian pharmaceutical preparations using DPPA on SMDE with negative amplitude di-sodium hydrogen phosphate 0.02 M buffer pH 8.5 according to the optimal conditions.

Commercial name	Label Claim of CLP, mg/tab.	*Mean ±SD (as CLP), mg/ tab.	RSD%	Assay %	* (Assay%), by HPLC [19]
Pharma GreL, F.C.Tablet **PHARMASYR,**	75	74.4	2.2	99.2	99.0
Plaraz, F.C. Tablet **AL–RAZI**	75	76.2	2.2	101.6	100.9
Norgrel Plus, F.C. Tablet, **UNIPHARMA**	75	75.6	2.1	100.8	101.0
Clopid, F.C. Tablet, **EL–SAAD**	75	74.2	2.2	98.9	99.5
Plofexine, F.C. Tablet, **ASIA,**	75	76.5	2.1	102.0	101.5
Clotless, F.C. Tablet, **APHAMEA**	75	76.3	2.1	101.7	101.8

***** n=5, Assay=(found mean/label claim)x100.

Method validation

The developed method for simultaneous estimation of CLPB has been validated in accordance with the International Conference on Harmonization guidelines (ICH) [22].

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 present at same amount with clopidogrel.

Linearity

Several aliquots of standard stock solution of CLPB were taken in different 25 mL volumetric flasks such that their final concentrations were 0.1050-12.5970 $\mu g.mL^{-1}$ of CLPB or 0.0805-9.6546 of CLP $\mu g.mL^{-1}$ (0.250-30.000 $\mu mol.L^{-1}$) for CLPB using DPPA at SMDE in di-sodium hydrogen phosphate 0.02 M buffer at pH 8.5. Linearity equation obtained was: $y = -12.662x - 0.1215$, for the mentioned range (R^2=0.9999).

Precision and Accuracy

The precision and accuracy of proposed method was 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 CLP. The basic concentration level of sample solution selected for spiking of the CLP standard solution was 5.1491 $\mu g.mL^{-1}$. The proposed method was validated statistically and through recovery studies, and was successfully applied for the determination of CLP in pure and dosage forms with percent recoveries ranged from 99.3% to 101.4 %, see Table 4.

Table 4: Results of recovery studies

Level	Recovery%
80%	99.3
100%	100.9
120%	101.4

(n=5).

Repeatability

The repeatability was evaluated by performing 10 repeat measurements for 5.1491 µg.mL^{-1}of CLP using the studied DPPA at SMDE di-sodium hydrogen phosphate 0.02 M buffer pH 8.5 under the optimum conditions. The found amount of CLP ($\bar{x}$±SD) was 5.1499±0.081 µg.mL^{-1} and the percentage recovery was found to be 100.02±1.6. These values indicate that the proposed method has high repeatability for CLP 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 and LOQ for CLP are 0.010 and 0.030 µg.mL^{-1}, 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 (8.5±0.20), and C_{elect} (0.02±0.002 mol.L^{-1}) and reaction waiting time (10 min), see Table 5. Indicates the robustness of the proposed method. I_p was measured and assay was calculated for five times.

Table 5: Robustness of the proposed DPPA method at SMDE for determination of clopidogrel.

Experimental parameter variation	Average recovery (%)* C_{CLP} =3.8618 µg.mL^{-1}
Temperature	
20^{o}C	99.5
25^{o}C	100.7
30 ^{o}C	101.0
pH	
8.3	99.9
8.7	100.0
$C_{Na_2HPO_4}$	
0.018 mol/L	99.8
0.022 mol/L	99.7
reaction time	
10 min	99.8
30 min	99.9
60 min	100.0

* n=5.

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 coformulated adjuvants.

The homogenization of tablets

The homogenization of tablets in terms of the weight and the amount of drug was studied. It found that the mean weight tablets were 0.2030 ± 0.0040 g (i.e. ±2.0%), 0.3068 ± 0.0053 g (i.e. ±1.7%), 0.6864± 0.0096 g (i.e. ±1.4%), 0.1850 ± 0.0040 g (i.e. ±2.2%), 0.4258 ± 0.0069 g (i.e. ±1.6%) and 0.2117 ± 0.0054 g (i.e. ±2.6%) for *Pharma Grel*, *Plaraz*, *Norgrel Plus*, *Clopid*, *Plofexine* and *Clotless* (75 mg/tab. of CLP). And amount of drug in the capsule was 75.00 ± 1.4 mg (i.e. ±1.9%), 75.00 ± 1.3 mg (i.e. ±1.7%), 75.00 ± 1.1 mg (i.e. ±1.5%), 75.00 ± 1.6 mg (i.e. ±2.1%), 75.00 ± 1.2 mg (i.e. ±1.6%), and 75.00 ± 2.0 mg (i.e.±2.7%), for the mentioned drugs, respectively; which shows that homogeneity of tablets is acceptable.

Conclusion

Electrochemical behavior and DPPA of CLPB in pure form and in pharmaceutical preparations using SMDE with 0.02 M di-sodium hydrogen phosphate buffer pH 8.5 according to the optimal conditions was applied. One reduction peak was observed. I_p is linear over the range 0.0805-9.6546 $\mu g.mL^{-1}$ (0.250-30.000 $\mu mol.L^{-1}$). The relative standard deviation did not exceed 2.2% for the concentration 0.0805 $\mu g.mL^{-1}$ of CLP. Regression analysis showed a good correlation coefficient (R^2=0.9999) between I_p and concentration over the mentioned range. The proposed method was successfully applied to the direct analysis of CLP in pharmaceutical formulations without any interference from excipients and with adequate accuracy and sensitivity without any pre-separation such as extraction.

References

1. Budavari S., 2011. "The Merck Index" 13th Ed. Merck & Co. Inc. 856.
2. Henein W., 2006. "Atlas 2 everything about drugs from A to Z". Nobar publisher. 282.
3. Alesci J.P., Victorino A., 2013. Clopidogrel: pharmacology, clinical uses and adverse effects, Nova Science Pub Inc; UK.
4. Mostafa A.M.A., 2016. clopidogrel personalization: Pharmacogenetics and pharmacometabonomics: A Review of the Literature, LAP Lambert Academic Publishing.
5. Anderson J.L., Morrow D.A., 2017. Acute myocardial infarction. The New England Journal of Medicine. 376(21):2053–2064.
6. Bin Ibrahim S.F., Alarfaj N.A., Aly F.A., 2012. "Determination of clopidogrel bisulfate using ion-selective electrodes in bulk, pharmaceutical formulation and in biological fluids". J.American Science. 8:276-283.
7. Khorshid A.F., 2014. "Determination of clopidogrel bisulphate in Plavix tablet and human biological fluids utilizing chemically modified carbon paste sensor". J. Bioproces Biotechniq. 4:1-9.
8. Haydar O.E., Khaleel A.I., Muhammed, K.O., 2019. "New electrodes for determination of clopidogrel- bisulphate".JUG.10: 55-69.
9. Dizavandi Z.R., Aliakbar A., Sheykhan M., 2017. "Electrochemical determination of clopidogrel using Bi_2O_3-Pp-AP/GCE by differential pulse voltammetry in pharmaceutical productions". J. Electroanalytical Chemistry. 17:1-31.
10. Mohammadi A., Barin S.M., Naeemy A., 2012. "Determination of clopidogrel using a graphite electrode modified by multi-walled carbon nanotube/ poly ortho aminophenol nanocomposite film". Research in Phamaceutical Science. 7:S645.
11. Nascimento L.O., Scremin J., Mattos G.J., Gomes A., Clausen D.N., Sartori E.R., 2019. "A Novel strategy for quantifying clopidogrel using square-wave voltammetry and a boron-doped diamond film". Electroanalysis. 31:1-8.
12. Mladenovic A.R., Jovanovic V.M., Petrovic S.D., Mijin D.Z., Drmanic S.Z., Ivic M.L., 2013. "Determination of clopidogrel using

square wave voltammetry at a gold electrode". J. Serb. Chem. Soc. 78:2131-2140.
13. Babaei A., Afrasiabi M., Moghanian H., 2017. "A new sensor based on the glassy carbon electrode modified with poly aspartic acid-Fe_3O_4 nanoparticle/ multi-walled carbon nanotubes composite for a selective simultaneous determination of piroxicam and clopidogrel in the presence of uric acid. Anal. Bioanal. Electrochem. 6: 741-761.
14. Malini S., Raj, K., Sennappan M., 2018."Electrochemical and spectroscopic studies of interaction of clopidogrel bisulphate with calf thymus DNA". Asian Journal of Chemistry. 30: 129-132.
15. Raj K., Malini S., 2017. "Cyclic voltammertic studies of *in vitro* interaction of clopidogrel bisulphate and aspirin". Materials Today. 5:22390-22398.
16. Abdul Sattar M.D., Rao U.U., Priyanka M., Kiran K.B., Sudha Ch.S., kumar G.V., 2014. "Method development and validation for the estimation of clopidogrel in tablet dosage form by UV spectrophotometric method". International Journal of Research and Novel Science. 1:171-175.
17. Mishra P., Dolly A., 2006. "Simultaneous determination of clopidogrel and aspirin in pharmaceutical dosage forms". J. Pharm. Sci. 68:365-368.
18. Ayad M.M., Abdellatef H.E., Hosny M.M., Sharaf Y.A., 2015. "Determination of pipazethate hydrochloride, fenoterol hydrobromide and clopidogrel hydrogen sulphate using citrate-capped gold nanoparticles". JCPR. 7: 68-74.
19. Shrivastava P.K., Basniwal P.K., Deepti J., Shrivastava S.K., 2008. "Concurrent estimation of clopidogrel bisulphate and aspirin in tablets by validated RP-HPLC Method". Indian J. Pharm. Sci. 70:667-669.
20. Sahoo N.K., Sahu M., Rao P,S., Indira J.N., Rani S.N., Ghosh G.K., 2014. "Validation of assay for bulk clopidogrel and for some tablet forms by reverse-phase high-performance liquid chromatography". J. Taibah Univ. Sci. 59:1-6.
21. Anderson J.E., Bond A.M., Jones R.D., 1981. "Differential Pulse Polarography at the Static Mercury Drop Electrode". Anal. Chem. 53 (7): 1016–1020.
22. ICH: Proceedings of the International Conference on Harmonization of Technical Requirement of Registration of Pharmaceuticals for Human Use (ICH Harmonized Tripartite Guidelines), 2005.

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Quick Response Code

DOI: 10.22192/ijcrcps.2020.07.05.001

How to cite this article:
Abdul Aziz Ramadan, Hasna Mandil, Nidal Ashram. (2020). Behavior and Quantification of Clopidogrel Bisulphate in Pure and Pharmaceutical Dosage Forms Using Differential Pulse Polarography With Static Mercury Drop Electrode. Int. J. Curr. Res. Chem. Pharm. Sci. 7(5): 1-13.
DOI: http://dx.doi.org/10.22192/ijcrcps.2020.07.05.001

