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Electrochemical study of the antiplatelet agent clopidogrel and its determination using differential pulse voltammetry in bulk form and pharmaceutical preparations with a glassy carbon electrode

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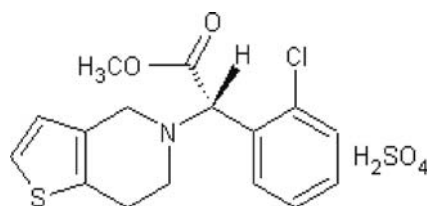
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In the present study, the electroanalytical behaviour of clopidogrel (CLP) bisulfate, an antithrombotic drug, was investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques using a glassy carbon electrode (GCE). The 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 in order to obtain the optimum oxidation conditions of this drug on a GCE. To determine the effects of the nature of the supporting electrolyte, pH and scan rate on the anodic oxidation reactions, the experiments were performed in 0.2 M sulphuric acid, and in Britton–Robinson (BR) (pH 2–5) and acetate (pH 3.5–5.63) buffers with a 10–400 mVs⁻¹ scan rate interval. The oxidation of clopidogrel bisulfate was found to be diffusion-controlled over a concentration range of 0.08 mM–1.0 mM in pH 3.7 acetate buffer by an optimized DPV technique. The voltammetric method developed was applied to the tablet form of pharmaceutical preparation of this compound and the accuracy, precision, selectivity, sensitivity, repeatability within and between days and reproducibility of the proposed method was investigated statistically. The results were compared with the spectrophotometric and HPLC methods developed in our laboratory and found to be in good agreement. No interference was observed from common pharmaceutical adjuvants.

1. Introduction

Clopidogrel is an oral antiplatelet agent (thienopyridine class) to inhibit blood clotting in coronary artery disease, peripheral vascular disease, and cerebrovascular disease. It is closely related to ticlopidine and appears to have a slightly more favorable toxicity profile with less frequent thrombocytopenia and leukopenia, although TTP (thrombotic thrombocytopenic purpura) has been reported (Herbert et al. 1993).

Clopidogrel hydrogen sulfate, methyl (+)-(S)- α -(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridin-5(4H)-acetate, is present in tablets as the hydrogen sulfate salt. All doses and tablet strengths are expressed as milligrams of base, not as hydrogen sulfate salt (Budavari 2001; Sweetman 2007; PDR® 2007).



Concentrations of antithrombotic drugs are quite low in body fluids at the therapeutic doses used, and sensitive analytical methods are necessary for their quantitative analysis (Harlfinger et al. 2004; Robinson et al. 2007; Mullangi and Srinivas 2009).

Therefore studies on this group of drugs and particularly studies of analytical techniques for clopidogrel have generally focused on chromatographic and spectrophotometric methods.

There are relatively few analytical studies relating to clopidogrel, a recently introduced antithrombotic drug. The analytical studies in the literature referring to clopidogrel, employ spectrophotometry (Mishra and Dolly 2005, 2006; USP XXX 2007, Zaazaa et al. 2009), HPLC (Mitakos and Panderi 2002; Singh et al. 2005; Soury et al. 2006; Gandhimathi and Ravi 2007), TLC (Antic et al. 2007), HPTLC (Agrawal et al. 2003), LC (Mitakos and Panderi 2002), LC-MS (Mitakos and Panderi 2004; Nirogi et al. 2006; Ksycinska et al. 2006; Shin 2007), GC-MS (Lagorce et al. 1998), LC-MS/MS (Takahashi et al. 2006, 2008), LC-ESI-MS/MS (Patel et al. 2008), and CZE (Fayet et al. 2009), analytical methods.

To the best of our knowledge, no electroanalytical methods have been used to determine this drug and its voltammetric characteristics have not yet been reported. No study concerned with examining the electrochemical structure of clopidogrel via electroanalytic methods, has yet been performed. Because no study examining clopidogrel oxidation-reduction by electrochemical methods exists, the method developed is original and has considerable importance for drug quantitation. In addition to spectroscopic and chromatographic methods recommended for analysis of clopidogrel in drug dosage forms and human plasma, electrochemical examination of the drug is also quite

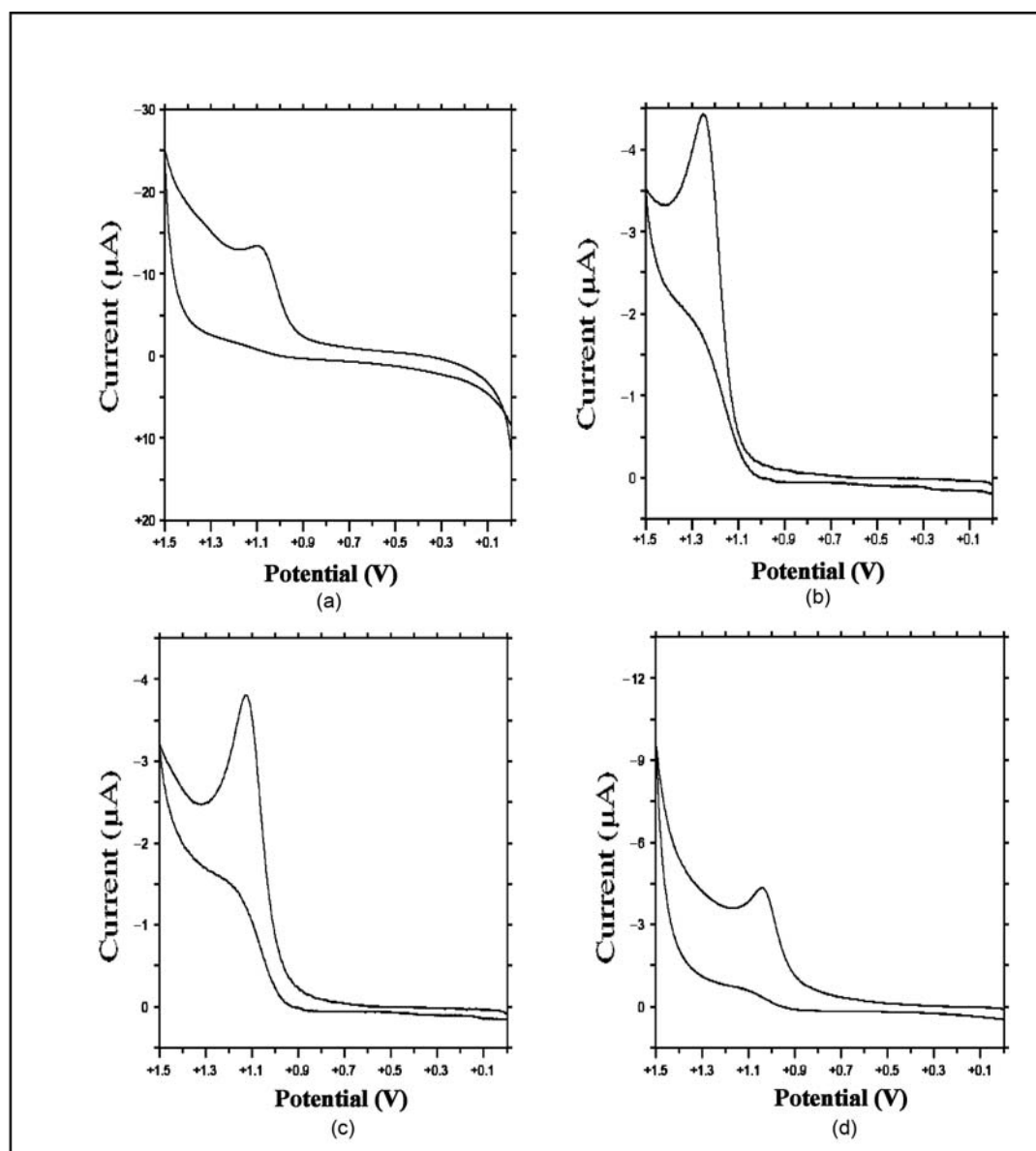


Fig. 1: Voltammograms of 5×10^{-4} M CLP obtained in
(a) 0.2 M H_2SO_4 supporting electrolyte;
(b) pH 1.9 BR buffer-methanol (1:1, v/v);
(c) pH 3 BR buffer-methanol (1:1, v/v);
(d) pH 3.7 acetate buffer-methanol (1:1, v/v)

important mechanistically and is complementary to them. This study aimed to examine clopidogrel electrochemically using a glassy carbon electrode and to elucidate its mechanism.

2. Investigations, results and discussion

Voltammetric techniques recommended for analysis of tablet dosage forms of drugs, provide an alternative method for drug quality control in view of their being sensitive, rapid, simple and easily applicable. DPV techniques were applied and the results were being evaluated from the viewpoint of quantitative analysis.

In this study the electrochemical behaviour on oxidation at a glassy carbon electrode of the antiplatelet drug clopidogrel was investigated using the DPV technique. The effects of supporting electrolyte type, pH, solution concentration and electrode on oxidation were examined.

Voltammetric experiments for oxidation of clopidogrel were carried out irreversibly at different pH values and with a variety

of buffer solutions. Voltammetric experiments were performed with 0.2 M H_2SO_4 supporting electrolyte, pH 1.9 BR buffer, pH 3 BR buffer and pH 3.7 acetate buffer (Fig. 1). It was concluded from these experiments that the optimum supporting electrolyte is pH 3.7 acetate buffer.

A glassy carbon electrode pretreated with mechanical cleaning, was used in the electrochemical experiments. Test results showed that this manner of cleaning the electrode surface is sufficient to ensure the repeatability of experiments.

CV curves were recorded over a wide range of scan rates ($10 - 400 \text{ mVs}^{-1}$). Cyclic voltammograms of 5×10^{-4} M CLP obtained in pH 3.7 acetate buffer (Fig. 2) showed one anodic peak at about 1000 mV. Voltammograms showing the effect of the scan rate are given in Fig. 2. As scan rate increased peak potentials shifted to more positive values and peak currents increased. When the different voltammograms were examined, it was found that speed increased linearly with current and the potential changed to a positive potential up to 68 mV. When the $i_p - \nu^{1/2}$ and $\log i_p - \log \nu$ relationships of different voltammogram of the drug, taken at $10 - 400 \text{ mVs}^{-1}$ scanning speeds with pH

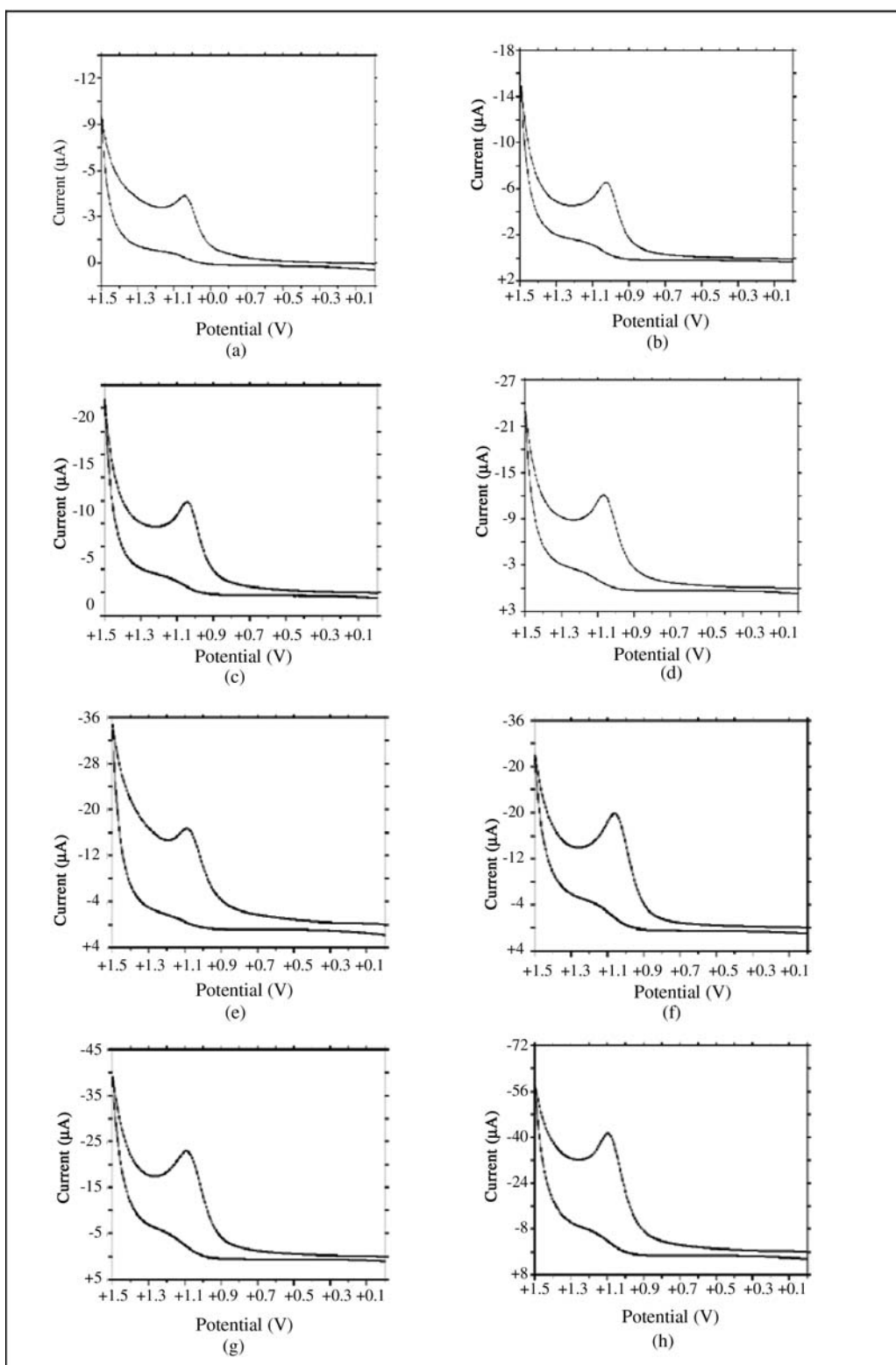


Fig. 2: Voltammograms of 5×10^{-4} M CLP obtained in pH 3.7 acetate buffer-methanol (1:1,v/v) at various scan rates.

(a) 10 mVs^{-1} , (b) 20 mVs^{-1} , (c) 40 mVs^{-1} , (d) 60 mVs^{-1} , (e) 80 mVs^{-1} , (f) 100 mVs^{-1} , (g) 200 mVs^{-1} , (h) 400 mVs^{-1}

3.7 acetate buffer, were examined, it was found that oxidation was diffusion-controlled. Regression analysis showed that the relationship between peak current and $\nu^{1/2}$ of voltammograms taken at different scanning speeds, with clopidogrel concentration 5×10^{-4} M in pH 3.7 acetate buffer solution, was linear. ($i_p = 1.5558 \nu^{1/2} - 2.1281$; $r^2 = 0.9910$, $n = 8$).

The straight line equation between logarithm of scanning speed and logarithm of peak current was found to be ($\log i_p = 0.44 \log \nu + 0.13$; $r^2 = 0.9989$, $n = 8$). Fig. 3 shows the influence of the

square root of the scan rate ($\log i_p - \log \nu$) also linear relationship. The slope in the equation being 0.44 demonstrated that the oxidation of clopidogrel was diffusion-controlled. In the literature it has been reported that the slope in diffusion-controlled electrochemical reactions is 0.5 (Laviron et al. 1980).

Fig. 4 gives differential pulse voltammograms obtained in acetate buffer solutions at different concentration of CLP. Evaluation of these curves revealed that quantitative determination of CLP could be made by DPV and the optimum conditions were

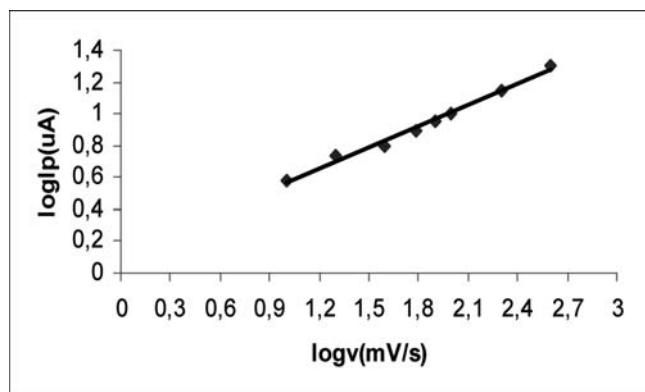


Fig. 3: Potential scan rate on the peak current-logarithm of scan rate ($\log i_p - \log v$) relationship

found to be 20 mVs^{-1} scan rate, 50 mV pulse amplitude, 17 ms sample width, 50 ms pulse width and 200 ms pulse period. Under these conditions the peak current of the DPV curve is linearly dependent on concentration.

When the voltammograms of the drug within a concentration range of $8 \times 10^{-5} - 1 \times 10^{-3} \text{ M}$ were taken in pH 3.7 acetate buffer solution using a glassy carbon electrode, linear relationship between current and concentration was found (Table 1). Statistical treatment of this dependence is given in Table 1. Reproducibility of DPV peak current and peak potential was tested by repeating ten experiments at $5 \times 10^{-4} \text{ M}$. The applicability of the DPV method for the assay of a simple dosage form was examined by analysis of a tablet form. The results confirm the suitability of the proposed method for the accurate and sensitive analysis of CLP. The validation of the procedures was carried out by evaluation of the limit of detection (LOD), limit of quantitation (LOQ), repeatability, recovery, specificity and robustness. The LOD and LOQ were calculated from the calibration curves as $k \times \text{SD}/b$ where $k = 3$ for LOD and 10 for LOQ, SD is the standard deviation of the intercept and b is the slope of the calibration curve (Miller and Miller 1984; USP XXVI 2003). The values of LOD and LOQ were $1.91 \times 10^{-5} \text{ M}$ (for DPV), $3.31 \times 10^{-6} \text{ M}$ (for spectrometry), $6.90 \times 10^{-7} \text{ M}$ (for HPLC) and $6.36 \times 10^{-5} \text{ M}$ (for DPV), $1.10 \times 10^{-5} \text{ M}$ (for spectrometry), $2.28 \times 10^{-6} \text{ M}$ (for HPLC), respectively. The quantitation limit of CLP achieved was low sufficiently. The intra-day and inter-day accuracy and precision results for CLP in bulk form are summarized in Table 2. Repeatability of the results was evaluated by performing six measurements at low, intermediate and high CLP concentrations in pH 3.7 acetate buffer on the GC electrode using the DPV technique. Mean percentage recoveries of $98.6 - 99.7\%$ with relative standard deviation of $0.87 - 1.96\%$ were found. The precision and accuracy of the proposed method

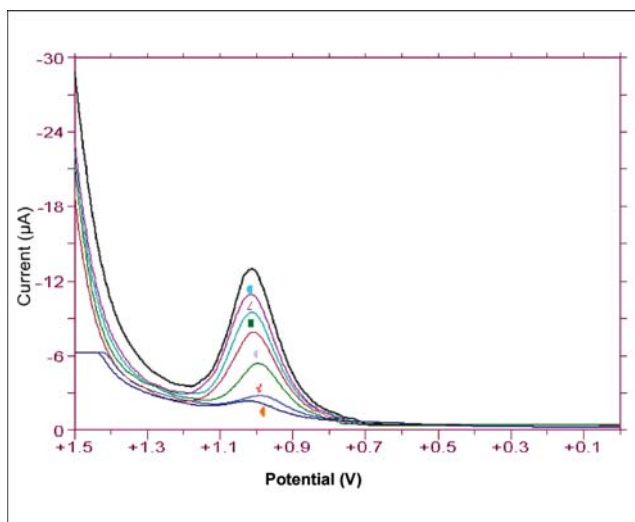


Fig. 4: DPV curves obtained in pH 3.7 acetate buffer: methanol (1:1,v/v) solution at various CLP concentration.

$\diamond \rightarrow 8 \times 10^{-5} \text{ M}$; $\star \rightarrow 1 \times 10^{-4} \text{ M}$; $\circ \rightarrow 2.5 \times 10^{-4} \text{ M}$;
 $\square \rightarrow 5 \times 10^{-4} \text{ M}$; $\triangle \rightarrow 7 \times 10^{-4} \text{ M}$; $\bullet \rightarrow 9 \times 10^{-4} \text{ M}$; $\blacktriangledown \rightarrow 1 \times 10^{-3} \text{ M}$

were investigated by intra- and inter-day determinations of CLP for three concentrations ($n = 6$) within the linear range. Accuracy of the method was expressed as bias % within and consecutive days was less than (-0.28%) at low and high concentrations.

The results revealed that any small change in the drug concentration of the solution can be accurately determined by this proposed method.

Results of voltammetric experiments performed for oxidation have shown that the chemical structure of CLP is electroactive. Voltammetric techniques for the active substance of CLP were also applied to Karum[®] film tablets, a pharmaceutical preparation of CLP. Film tablet solutions with appropriate concentration were prepared and voltammograms were taken under the same experimental conditions (Fig. 5). Test results demonstrate that the excipients in the tablet are not electroactive and do not affect the voltammetric analysis. It was concluded that the voltammetric method developed was considerably specific for clopidogrel active substance (Fig. 5). The results of the tests performed demonstrated that the voltammetric techniques developed are applicable directly, easily and rapidly to clopidogrel tablet dosage forms, and that excipients which are present in addition to the active substance do not have electrochemical activities. This study showed that the DPV technique developed for analysis of CLP fulfills the requirement to be sensitive, reliable, accurate, selective, simple and rapid without being affected by excipients and without the necessity for sample pretreatment or time-consuming extraction steps prior to analysis.

Table 1: Determination of CLP by DPV, UV-spectrophotometry and HPLC

Parameters	DPV	UV-Spectrophotometry	HPLC
Range (M)	$8 \times 10^{-5} - 1 \times 10^{-3}$	$4 \times 10^{-5} - 1 \times 10^{-3}$	$3 \times 10^{-6} - 1.8 \times 10^{-5}$
Regression equation (Y) ^a			
Slope (b)	9.3476×10^3	663.15	2356.8
S.D. of slope (S_b)	0.5350×10^3	304.09	429.15
Intercept (a)	0.786	0.007	252.74
S.D. of intercept (S_a)	0.328	0.112	210.25
Regression coefficient (r^2)	0.998	0.999	0.999
LOD (M)	1.91×10^{-5}	3.31×10^{-6}	6.90×10^{-7}
LOQ (M)	6.36×10^{-5}	1.10×10^{-5}	2.28×10^{-6}

^a $Y = a + bC$ where C is concentration in M and Y in absorbance, current and peak area units for UV- spectrophotometric, voltammetric and HPLC methods, respectively

Table 2: Analytical precision and accuracy for clopidogrel determination in bulk form by proposed DPV voltammetric procedure

Method	Inter-day (n = 6)					
	Concentration (taken) (M)	Concentration (found) (M)	Recovery %	S.D.	Precision R.S.D. %	Accuracy bias %
DPV	2.5×10^{-4}	2.49×10^{-4}	99.6	0.075	2.41	−0.4
	5×10^{-4}	4.92×10^{-4}	98.4	0.073	1.35	−1.6
	7×10^{-4}	6.96×10^{-4}	99.4	0.634	0.86	−0.57
		X_{mean}	99.13			
		S.D.	0.643			
		R.S.D.	0.65			
intra-day (n = 6)						
DPV	2.5×10^{-4}	2.47×10^{-4}	98.8	0.061	1.96	−1.2
	5×10^{-4}	4.93×10^{-4}	98.6	0.103	1.91	−1.4
	7×10^{-4}	6.98×10^{-4}	99.7	0.065	0.87	−0.28
		X_{mean}	99.03			
		S.D.	0.585			
		R.S.D.	0.59			

X_{mean} : Mean value. S.D.: Standard deviation. R.S.D.(%): Relative standard deviation. Inter-day: Consecutive days. Intra-day: Within one day.

Table 3: Comparative studies for clopidogrel formulations

Formulation ^a (tablet)	Analysis techniques		
	Voltammetry (DPV)	UV- Spectrophotometry	HPLC
Mean (mg) ^b	74.97	74.88	75.24
R.S.D. (%) Calculated t value	0.22 ^c	0.23 ^c	
T , theoretical ($p=0.05$)			

^a Tablet, 75 mg per tablet

^b Each value is the mean of six experiments

^c NS, not significant

To show the accuracy of the voltammetric technique developed, this analytical method (DPV), which is also applicable to the tablet dosage form of the drug, were compared to the results of drug analysis by UV-spectrophotometric and high pressure liquid chromatography methods. Statistical studies demonstrated that there is no difference in sensitivity between the methods for drug analysis (Table 3). The applicability of the proposed voltammetric method to the assay of simple dosage forms was

examined by analyzing tablets. The voltammetric results were compared with the spectrophotometric (USPXXX, 2007) and chromatographic results by Student's t-test at a 95% confidence level, and no significant difference was found between them (Table 3). The analytical results in this study demonstrated that the amount of active substance in the drug is within limits specified in the pharmacopoeia. The proposed method was developed as an alternative to chromatographic and spectrophotometric methods, and the results obtained were promising. It was demonstrated that the voltammetric method developed could be applied easily, accurately and sensitively to pharmaceutical dosage forms. The t values obtained in the comparison of methods (0.22 for calculated DPV – HPLC, 0.23 for UV spectrophotometry – HPLC) were smaller than the tabulated t value (2.57), so the difference was found to be non-significant. It will be seen that the results obtained are compatible within the confidence interval. These results exhibit the accuracy of the voltammetric technique developed. The DPV method may be recommended for routine and quality control analysis of the drug investigated in pharmaceutical dosage forms.

3. Experimental

3.1. Apparatus

Voltammetric measurements were made using a BAS 100 W/B electrochemical analyzer. The three-electrode system comprised a BAS MF 2012 glassy carbon disc working electrode, a BAS MF 1063 type silver/silver chloride/saturated KCl reference electrode and a BAS MV 1032 platinum wire auxiliary electrode. A double beam, Shimadzu model 1601 spectrophotometer with a fixed slit width (2nm) connected to an IBM-PC computer was used. The UV spectra of standard and test solutions were recorded in 1 cm quartz cells.

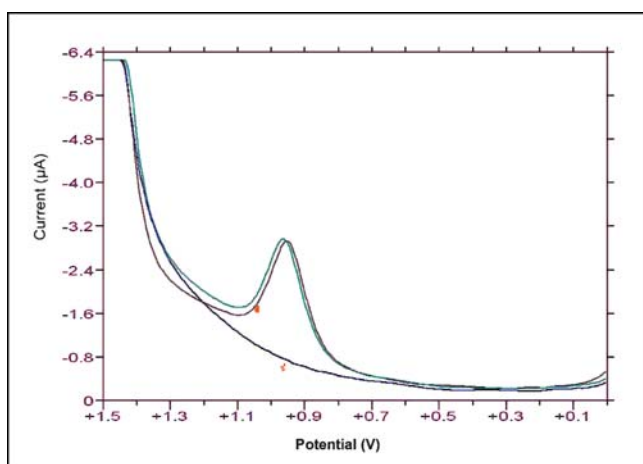


Fig. 5: Voltammograms of 1×10^{-4} M CLP (bulk form and tablet formulations) in supporting electrolyte (pH 3.7 acetate buffer: methanol, 1:1,v/v).

—: Pure drug in supporting electrolyte;

- - - : Supporting electrolyte;

... : Drug in tablet formulations in supporting electrolyte

An Agilent 1100 liquid chromatograph equipped with a G13 79A degasser, 613 11A quaternary pump, G13 13A auto sampler, and 61315B DAD detector was used for chromatographic measurements. The chromatograms were recorded and the peaks were quantitated using the automatic integrator. The chromatograms were carried out at a temperature of 40 °C on a Nova-Pak® C18 column of 3.9 × 150 mm (4 µm particle size).

3.2. Reagents

Clopidogrel is marketed as clopidogrel bisulfate (clopidogrel hydrogen sulfate), most commonly under the trade name Plavix®, as 75 mg oral tablets. CLP was obtained from Sanovel without prior purification. Karum® tablets containing a 75 mg dose were obtained from local pharmacies. Karum tablets are available as a tablet forms with 75 mg clopidogrel equivalent to 79 mg clopidogrel bisulfate. For the required purity control of clopidogrel active substance, its melting point was determined and it was found to have sufficient purity by obtaining UV and IR spectra. Analytical grade phosphoric acid, and Merck grade methanol were purchased from Merck & Co. All other chemicals were of analytical-reagent grade and were used as received.

3.3. Solution preparation

A stock solution of 10^{-3} M CLP was prepared by dissolving 42 mg CLP in 50 mL of methanol and diluting it to 100 mL with acetate buffer (pH 3.7). Working standard solutions were prepared daily by appropriate dilution of the stock solution with acetate buffer. BR buffers were prepared according to the literature (Britton 1952). Acetate and phosphate buffers were prepared according to USP pharmacopoeial procedure.

3.4. Pretreatment of the working electrode

The glassy carbon electrode was polished with 0.5 µm alumina powder on a polishing cloth prior to each measurement. Then it was thoroughly rinsed with methanol and double distilled water, and gently dried with a paper tissue.

3.5. Procedure

Stock solutions of concentrations 8×10^{-5} – 1×10^{-3} M, 4×10^{-5} – 1×10^{-3} M and 3×10^{-6} – 1.8×10^{-5} M were prepared in methanol: pH 3.7 acetate buffer (1:1, v/v), 0.1 N HCl and pH 8 phosphate buffer: acetonitrile (30:70, v/v) and stored in dark bottles at +4 °C. The working solutions for voltammetric, spectrophotometric and chromatographic investigations were prepared by dilution of the stock solution. The CLP concentration did not change with time. All working solutions were prepared freshly every day.

3.6. UV Spectrophotometry

Absorption spectra of CLP in 0.1 N HCl were determined by zero-order spectrophotometry of this drug in tablet forms. For determination of CLP, measurement of the peak-zero amplitude in the zero-order spectra at 270 nm was used.

3.7. HPLC

The mobile phase was pH 8 phosphate buffer: acetonitrile (30:70, v/v). The flow rate was set at 0.8 mL/min with 10 µL injection volume and the detection wavelength was 210 nm for chromatographic measurements.

3.8. Analysis of tablets

3.8.1. Voltammetric method

A commercial pharmaceutical preparation was assayed. Twenty tablets of CLP (each containing 75 mg CLP) were accurately weighed and finely powdered. The correct amount of powder was dissolved in 50 mL methanol and then acetate buffer (pH 3.7) supporting electrolyte were added and made up to 100 mL. After 10 min of ultrasonic shaking, 5 mL of this solution was filtered through 0.45 µm membrane in a 50 mL volumetric flask. The volume was made up to 50 mL with the acetate buffer (pH 3.7) supporting electrolyte. All the test solutions were obtained by diluting this stock solution with the selected supporting electrolyte. The voltammograms were recorded following the voltammetric procedure already outlined.

3.8.2. Spectrophotometric method

Twenty tablets of CLP were weighed and thoroughly powdered. The average of one tablet was weighed out, transferred into a 50 mL volumetric flask and dissolved in 0.1 N HCl; the flask was left in an ultrasonic bath for 5 min. After 5 min of ultrasonic shaking, 5 mL of this solution was filtered through

a 0.45 µm membrane filter. The volume was made up to 50 mL with 0.1 N HCl. All the test solutions were obtained by diluting this stock solution with 0.1 N HCl. UV spectra were then recorded.

3.8.3. Chromatographic method

Twenty tablets of CLP were weighed and thoroughly powdered. The average of one tablet was weighed out transferred into a 100 mL volumetric flask and dissolved in the mobile phase (pH 8 phosphate buffer: acetonitrile 30:70, v/v). The flask was left in an ultrasonic bath for 10 min. After 10 min of ultrasonic shaking, 1 mL of this solution was filtered through a 0.45 µm membrane filter. The volume was made up to 100 mL with the mobile phase. All the test solutions were obtained by diluting this stock solution with the mobile phase and chromatograms were recorded.

References

- Agrawal H, Kaul N, Paradkar AR, Mahadik KR (2003) Stability indicating HPTLC determination of clopidogrel bisulphate as bulk drug and in pharmaceutical dosage form. *Talanta* 61: 581–589.
- Antic D, Filipic S, Agbaba DD (2007) A simple and sensitive TLC method for determination of clopidogrel and its impurity SR 26334 in pharmaceutical products. *Acta Chromatogr* 18: 199–206.
- Britton HTS, in: *Hydrogen ions*, 4th ed., Chapman & Hall, London, (1952), p. 113.
- Budavari S, eds., in: *The Merck Index*, 13th ed., Merck & Co., Inc., Whitehouse Station, NJ, (2001), p. 856.
- Fayed AS, Weshahy SA, Shehata MA, Hassan NY, Pauwels J, Hoogmartens J, Schepdael A (2009) Separation and determination of clopidogrel and its impurities by capillary electrophoresis. *J Pharm Biomed Anal* 49: 193–200.
- Gandhimati M, Ravi TK (2007) High performance liquid chromatographic determination of aspirin and clopidogrel. *Ind J Pharm Sci* 69: 123–125.
- Harlfinger S, Berkels R, Tauber D, Beckerath NV, Gorchakova O, Kasrati A, Schömig E (2004) Pharmacokinetics of clopidogrel and its active metabolite in human plasma. *J Am Coll Cardiol* 43 supplement 2,3, p. A492.
- Herbert JM, Frehel D, Vallee E, Kieffer G, Gouy D, Berger Y, Necciari J, Defreyn G, Maffrand JP (1993) Clopidogrel; a novel antiplatelet and antithrombotic agent. *Cardiovasc Drug Rev* 11: 180–198.
- Ksycinska H, Rudzki P, Bukowska-Kiliszek M (2006) Determination of clopidogrel metabolite (SR 26334) in human plasma by LC-MS. *J Pharm Biomed Anal* 41: 533–539.
- Laviron E, Roulier L, Degrand C (1980) A multiplayer model for the study of space distributed redox modified electrodes: Part II theory and application of linear potential sweep voltammetry for a simple reaction. *J Electroanal Chem* 112: 11–23.
- Lagorce P, Perez Y, Ortiz J, Necciari J, Bressole F (1998) Assay method for the carboxylic acid metabolite of clopidogrel in human plasma by gas chromatography mass-spectrometry. *J. Chromatogr B: Biomed Sci and Appl* 720: 107–117.
- Miller JC, Miller JN (1984) in: *Statistics for Analytical Chemistry*, 2nd ed. Wiley, New York, pp. 83–117.
- Mishra P, Dolly A (2005) Spectrophotometric methods for determination of clopidogrel in tablets. *Indian J Pharm Sci* 67: 491–493.
- Mishra P, Dolly A (2006) Simultaneous determination of clopidogrel and aspirin in pharmaceutical dosage forms. *Indian J Pharm Sci* 68: 365–368.
- Mitakos A, Panderi I (2002) A validated LC method for the determination of clopidogrel in pharmaceutical preparations. *J Pharm Biomed Anal* 28: 431–438.
- Mitakos A, Panderi I (2004) Determination of the carboxylic acid metabolite of clopidogrel in human plasma by liquid chromatography-electrospray ionization mass spectrometry. *Anal Chim Acta* 505: 107–114.
- Mullangi R, Srinivas NR (2009) Clopidogrel: review of bioanalytical methods, pharmacokinetics/pharmacodynamics and update on recent trends in drug-drug interaction studies. *Biomed Chromatogr* 23: 26–41.
- Nirogi RVS, Kandikere VN, Shukla M, Mudigonda K, Maurya S, Boosi R (2006) Quantification of clopidogrel in human plasma by sensitive liquid chromatography/tandem mass spectrometry *Rapid Commun Mass Spectrom* 20: 1695–1700.
- Patel NK, Subbaiah G, Shah H, Kundlik M, Shrivastav PS (2008) Rapid LC-ESI-MS-MS method for the simultaneous determination of clopidogrel and its carboxylic acid metabolite in human plasma. *J Chromatogr Sci* 46: 867–875.

- Physicians, Desk Reference (PDR®) (2007) 61st ed. pp. 2926–2929.
- Robinson A, Hillis J, Neal C, Leary AC (2007) The validation of bioanalytical method for the determination of clopidogrel in human plasma. *J Chromatogr B Anal Tech Biomed Life Sci* 848: 344–354.
- Shin BS, Yoo SD (2007) Determination of clopidogrel in human plasma by liquid chromatography/tandem mass spectrometry: application to a clinical pharmacokinetic study. *Biomed Chromatogr* 21: 883–889.
- Singhs SS, Sharma K, Barot D, Mohan PR, Lohray VB (2005) Estimation of carboxylic acid metabolite of clopidogrel in Wistar rat plasma by HPLC and its application to pharmacokinetic study. *J Chromatogr B Anal Tech Biomed Life Sci* 821: 173–180.
- Souri E, Jalalizadeh H, Kebriaee-Zadeh A, Shekarchi M, Dalvandi A (2006) Validated HPLC method for determination of carboxylic acid metabolite of clopidogrel in human plasma and its application to pharmacokinetic study. *Biomed Chromatogr* 20: 1309–1314.
- Sweetman SC, ed. (2007) *Martindale: The Complete Drug Reference* The Pharmaceutical Press, (London) (35): pp.1424–1425.
- Takahashi M, Pang, H, Kikuchi A, Kawabata K, Kurihara A, Farid AN, Ikeda T (2006) Stabilization of the clopidogrel active metabolite in whole blood and its assay in human plasma by LC-MS/MS. *Proceedings of the 54th ASMS Conference on Mass Spectrometry and Allied Topics*, May 28–June 1, 2006, Seattle, WA, USA.
- Takahashi M, Pang H, Kawabata K, Farid NA, Kurihara, A (2008) Quantitative determination of clopidogrel active metabolite in human plasma by LC-MS/MS. *J Pharm Biomed Anal* 48: 1219–1224.
- The United States Pharmacopoeia, in: *Validation of Compendial Methods*, (2003), 26th ed, Pharmacopoeial Convention Inc., Rockville, MD, pp. 2439–2442.
- The United States Pharmacopoeia, Thirtieth Revision, and The National Formulary, (2007), 25th ed., Rockville, USA, pp. 1802–1805.
- Zaazaa HE, Abbas SS, Abdelkawy M, Abdelrahman MM (2009) Spectrophotometric and spectrodensitometric determination of clopidogrel bisulfate with kinetic study of its alkaline degradation. *Talanta* 78: 874–884.