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Electrochemical Study of Clopidogrel and its Determination in Pharmaceutical Preparations Using Square Wave Voltammetry

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ABSTRACT

In the present study, the electroanalytical behaviour of clopidogrel was investigated by cyclic voltammetry method. The procedure was based on clopidogrel being electrochemically oxidized at a platinum electrode in nonaqueous solutions. At 1.93 V, the oxidation peak was noted. It was discovered that clopidogrel's oxidation was diffusion-controlled. Additionally, a quick and easy SWV approach was developed and validated in this work to determine clopidogrel in pharmaceutical preparations. At concentrations between 5 and 50 µg/mL, the calibration curve was linear. The precision was given by relative standard deviation and was less than 2.73%. Accuracy was given with relative error and did not exceed 3.89%. In pharmaceutical preparations, clopidogrel had an average recovery of 100.1%. Under the chosen experimental conditions, no interference was found. The suggested method is extremely accurate and precise. Therefore, the method is applicable to the measurement of clopidogrel in pharmaceutical formulations.

Keywords: Clopidogrel, Voltammetry, Validation, Analysis.

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Introduction

Antithrombotic medications are prescribed to stop thrombosis. Thrombosis, which is the development of blood clots in veins, can be lethal for individuals. These medications lessen the chance of blood clotting. Due to their preventive qualities against cardiovascular disorders, antithrombotic medicines are one of the pharmacological classes that have recently been investigated [1–7].

Drugs that contain "clopidogrel" as a medication ingredient are used therapeutically to lower myocardial infarction risk, atherothrombotic disorders, and cardiovascular diseases [8,9]. The molecular name of clopidogrel is 4,5,6,7-tetrahydrothieno[3,2-c] pyridine (Figure 1). After examining its effects in more than 30000 patients worldwide, the clinical advantage of long-term clopidogrel treatment in reducing atherothrombotic disorders is demonstrated [10,11].

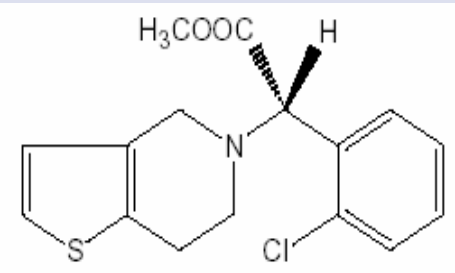

Figure 1. Structure of clopidogrel.

Reviews of the literature indicate that UV spectrophotometry [12], high-performance liquid chromatography with ultraviolet detection [13], gas chromatography with mass spectrometry [14], and high-performance liquid chromatography with mass spectrometry [15,16] are used to quantitatively analyze clopidogrel in pharmaceutical preparations or biological liquids.

There are many chromatographic methods for determining the presence of clopidogrel in human plasma, according to a thorough literature review. Endogenous interference, possible drug loss during re-extraction, arduous and time-consuming plasma sample preparation and extraction processes, and the necessity for sophisticated and expensive equipment all had an impact on the reported techniques.

It is crucial to develope a new technique for figuring out how much medication is in pharmaceutical dosage forms. Numerous pharmaceutical substances have been identified utilizing electroanalytical techniques, which have the benefits of not typically requiring derivatization and being less susceptible to matrix effects than other analytical techniques. The identification of electrode mechanism is another electrochemistry application. Drugs' redox characteristics can provide information about their pharmacological efficacy, in vivo redox activities, or metabolic destiny.

Although the electrochemical behavior and oxidation mechanism of clopidogrel have analytical significance, no

research on the voltammetric oxidation of clopidogrel in nonaqueous fluids has been published. It is widely known that the electrochemical process and voltammetric response of pharmaceuticals are directly influenced by the experimental and operational parameters. So, it would be interesting to look at how clopidogrel oxidizes in aprotic environments. However, the voltammetry method has not yet been used to quantitatively assess clopidogrel using a platinum electrode. The major goal of this work was the development of a novel SWV technique for the rapid and precise evaluation of clopidogrel in pharmaceutical preparations without the necessity for laborious extraction or evaporation procedures prior to drug testing.

This study describes SWV methods using a platinum disc electrode to determine clopidogrel using simple, quick, and selective processes that have been completely verified. Also, the technique was effectively used to evaluate the consistency of the formulation content and to quantitate a commercially available clopidogrel medication for QC.

Materials and Methods

Chemicals

Clopidogrel bisulfate standard (98≥ purity), *lithium perchlorate* ($LiClO_4$) and acetonitrile were purchased from Sigma (Germany). Plavix and Karum tablets that included 75 mg clopidogrel were purchased from a pharmacy (Erzurum, Turkey).

Electrochemical Instrumentation

Using the software PHE 200 and PV 220, electrochemical experiments were carried out on a Gamry Potentiostat Interface 1000. The single-compartment electrochemical cell used for all tests has a conventional three-electrode setup. Platinum wire served as the counter electrode and a platinum disk served as the working electrode. On microcloth pads, 1.0, 0.3, and 0.05 µm alumina slurries were used to incrementally polish the working electrode. The reference electrode for each potential was made of Ag/AgCl/KCl (3.0 M). The potential was cycled between 1.7 and 2.1 V at a sweep rate of 0.1 V/s. The SWV was operated at pulse amplitudes of 25 mV, 10 Hz, 4 mV potential step and 0.1 V/s scan rate.

Preparation of Standard Solutions

In 0.1 M $LiClO_4$/acetonitrile, the stock standard solution of clopidogrel (100 µg/mL) was prepared. This stock solution was used to prepare working standard solutions. The concentrations of the standard solutions were 5, 10, 15, 20, 25, 30, 40, and 50 µg/mL. The QC solutions were created at concentrations of 7.5, 27.5, and 45 µg/mL.

Results and Discussion

Development and Optimization of the Method

The electrochemical behavior of clopidogrel was investigated at the Pt disc electrode. An acetonitrile solution containing 0.1 M $LiClO_4$ was used as the supporting electrolyte in cyclic voltammetry. Figure 2 depicts a typical cyclic voltammogram for 100 µg/mL clopidogrel at 0.1 V/s scan rate. The oxidation peak was seen in the anodic sweep at 1.93 V.

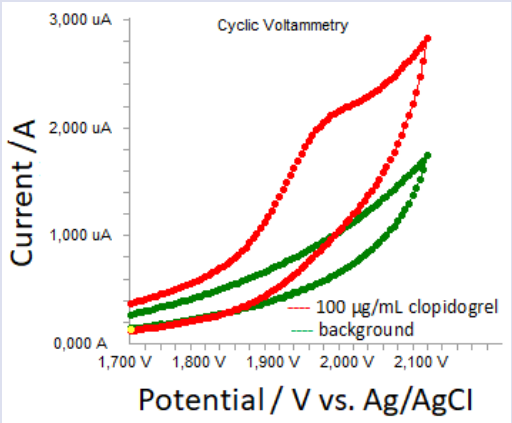

Figure 2. Cyclic voltammogram of clopidogrel (100 µg/mL)

The influence of scan rate on the anodic peak currents and peak potentials was investigated in the range of 0.01-1 V/s of the potential scan rates in 0.1 M $LiClO_4$/acetonitrile solution containing clopidogrel in order to better comprehend the voltammetric waves (Figure 3).

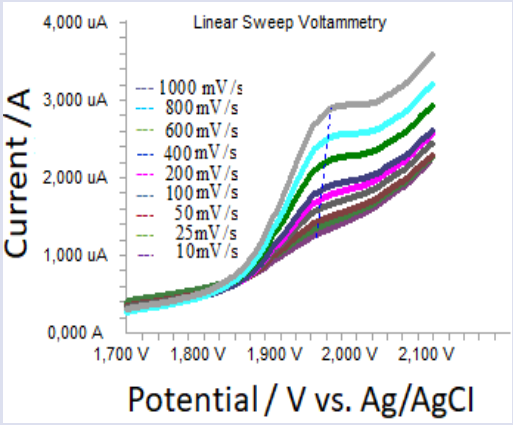

Figure 3. Linear sweep voltammograms for the oxidation of 30 µg/mL clopidogrel as a function of scan rate (10, 25, 50, 100, 200, 400, 600, 800 and 1000 mV/s)

Figure 4a,b shows the linear sweep voltammograms for clopidogrel as a function of scan rate. However, at clopidogrel concentrations of 30 µg/mL, the logarithm of peak currents against logarithm of scan rates graphs display straight lines with a slope of 0.36 (Figure 4c), which is close to the predicted value of 0.5 anticipated for an ideal diffusion-controlled electrode process [17].

In order to accomplish this, the *log I-log v* curve is more suitable, therefore a diffusional process for the peak should be taken into account. These findings show that the redox species are readily diffusing from the solution as opposed to precipitating onto the electrode surface. This phenomenon can be brought on by either a

lack of product adhesion to the electrode surface or the solubility of the intermediate species in acetonitrile.

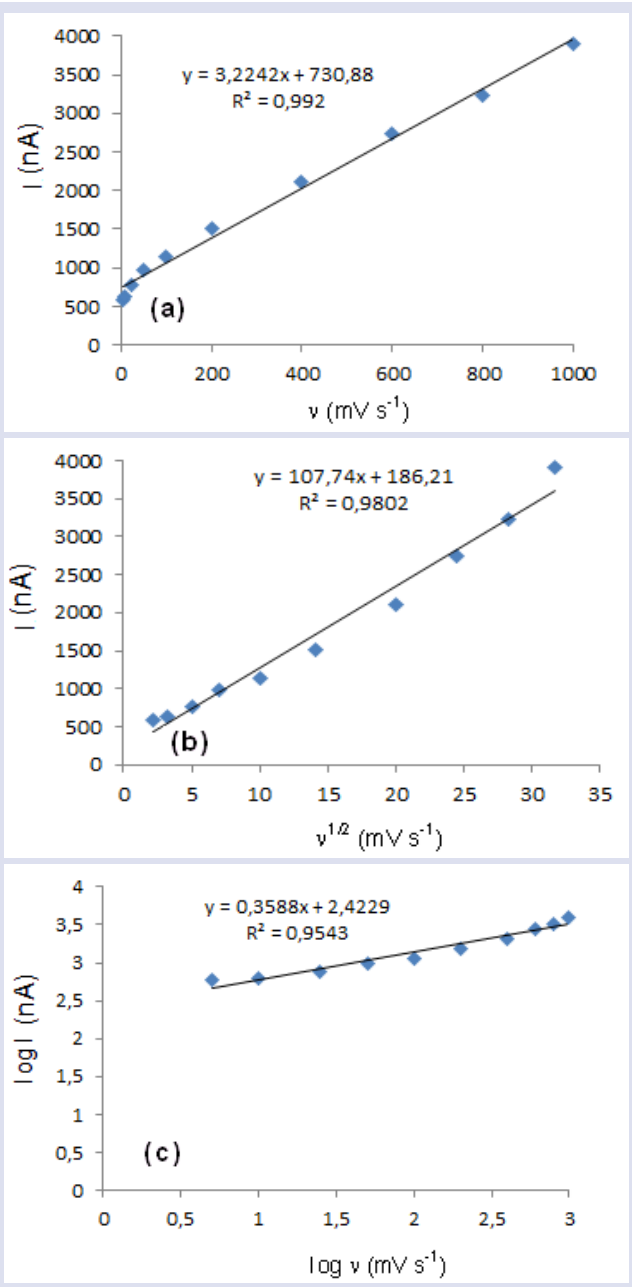

Figure 4(a-c). Peak current dependence on scan rate (30 µg/mL).

Figure 3 shows the movement of the oxidation peak potential *(E_{pa})* for peaks toward higher positive values as the scan rate is increased. The equation below [18] describes the relationship between the peak potential and scan rate,

$$E_{pa} = E^{0'} + RT/\left[(1-\alpha)n_a F\right]\left[0.78 + \ln\left(D^{1/2}k_s^{-1}\right) - 0.5\ln RT/\left[(1-\alpha)n_a F\right]\right] + RT/\left[(1-\alpha)n_a F\right]/2\ln v$$

and from the variation of peak potential with scan rate αn_a can be determined, where α is the transfer coefficient and n_a is the number of electrons transferred in the rate determining step. The plots of the oxidation peak potentials against *ln v* demonstrate a linear connection in accordance with this equation (Figure 5).

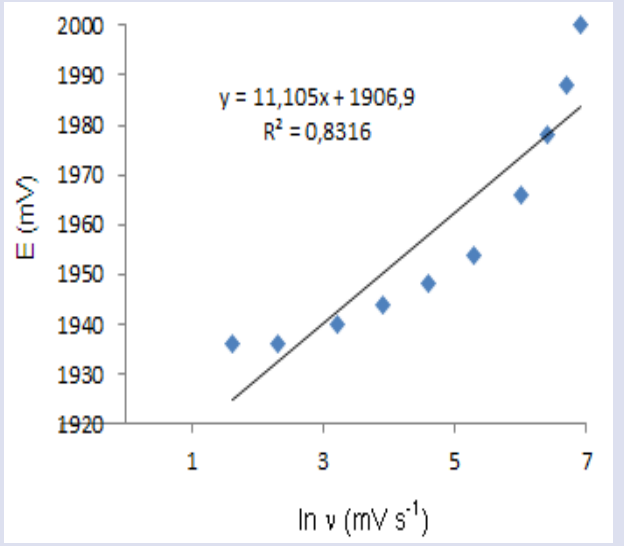

Figure 5. Dependence of the clopidogrel anodic peak potentials on the scan rate.

The slope indicates that the highest value of αn is 11.105. Additionally, this value shows that the processes of electron transfer are completely irreversible. This outcome demonstrates that the chemical step is a charge transfer and a quick following reaction.

Validation of the Method

ICH Q2B guidelines were followed while determining the validation parameters [19]. These criteria include specificity, linearity, precision, accuracy, recovery, limit of detection (LOD), limit of quantification (LOQ), robustness and stability.

Specificity

In this study, it was investigated the potential interferences of common excipients and additives. The control samples were prepared and examined. At the concentrations present in dosage forms, there is no evidence of any interference from these chemicals. The excipient employed in this formulation was one that the pharmaceutical industry employs most frequently. The method's specificity was examined by keeping an eye out for any interference from common tablet ingredients like talc, lactose, sodium chloride, titanium dioxide, and magnesium stearate. These exceptions had no negative effects on the suggested method. The procedure might be specific in accordance with the findings of the analysis.

Linearity

Standard solutions at concentration of 5, 10, 15, 20, 25, 30, 40 and 50 µg/mL were prepared for SWV (Figures 6). Plotting the clopidogrel concentration versus peak current responses allowed for the construction of the calibration curve for the clopidogrel (Fiure 7).

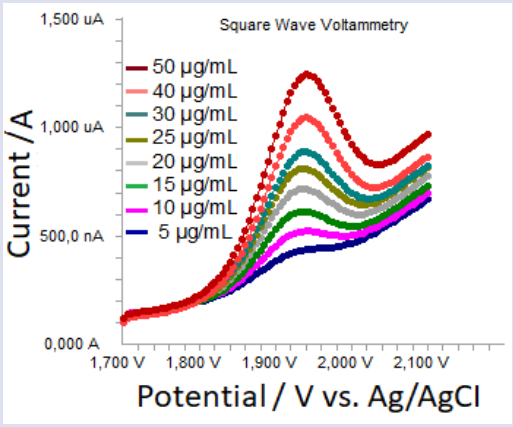

Figure 6. SWV voltammograms of clopidogrel

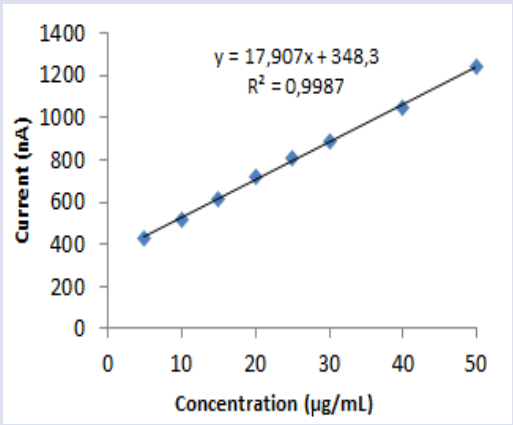

Figure 7. Calibration curve of clopidogrel (5, 10, 15, 20, 25, 30, 40 and 50 µg/mL).

All of the calibration curves' correlation coefficients (r) were consistently higher than 0.99. Using the least squares method and the Microsoft Excel® application, the linear regression equations were derived and described in Table 1.

Table 1. Linearity of clopidogrel

Parameters	Clopidogrel
Linearity range (µg/mL)	5-50
Slope	17.907
Intercept	348.3
Correlation coefficient	0.9987
LOD (µg/mL)	1.50
LOQ (µg/mL)	4.50

Precision and accuracy

Using the QC samples, the SWV method's precision and accuracy were assessed for intra-day and inter-day. The same-day analysis of the QC samples served to assess intra-day precision and accuracy. It was able to assess the precision and accuracy between days by contrasting the assays performed on two distinct days. The intra-day accuracy ranged from 1.11% to 2.54%, while the precision ranged from 0.95% to 3.06% (Table 2). It is evident from the results that this process has good accuracy and precision.

Table 2. Precision and accuracy of clopidogrel

Added (µg/mL)	Intra-day Found ± SD[a]	Intra-day Precision % RSD[b]	Intra-day Accuracy[c]	Inter-day Found ± SD[a]	Inter-day Precision % RSD[b]	Inter-day Accuracy[c]
7.5	7.4 ± 0.142	1.92	-1.33	7.6 ± 0.205	2.70	1.33
27.5	26.8 ± 0.821	3.06	-2.54	27.2 ± 0.914	3.36	-1.09
45	44.5 ± 0.424	0.95	-1.11	45.9 ± 0.532	1.16	2.00

Recovery

At three different concentrations, the recovery was examined to investigate the impacts of formulation interference. The recoveries were carried out by mixing pre-analyzed samples of clopidogrel tablets with a known quantity of pure medicines. The recoveries were calculated by comparing the amounts extracted from the spiked samples with the actual added concentrations. The results are listed in Table 3.

Table 3. Recovery of clopidogrel in tablets (n=6)

Tablet	Added (µg/mL)	Found ± SD	%Recovery	%RSD
Plavix (20 µg/mL)	5	4.9 ± 0.131	98.0	2.67
	15	14.9 ± 0.312	99.3	2.09
	25	25.3 ± 0.684	101.2	2.70
Karum (20 µg/mL)	5	4.9 ± 0.107	98.0	2.18
	15	14.7 ± 0.362	98.0	2.46
	25	25.2 ± 0.439	100.8	1.74

LOD and LOQ

The suggested technique's LOD and LOQ values were calculated using calibration standards. LOD and LOQ values were calculated as 3.3 /S and 10 /S, respectively. In this equation, S is the calibration curve's slope and is the y-intercept's standard deviation (n=6). The results are summarized in Table 1.

Ruggedness

The same instrument and standard standard solution were used in this study by a separate analyst to assess the concentration of clopidogrel (Table 4). No statistically significant discrepancies between the operators were found in the results, indicating the ruggedness of the developed approach.

Table 4. Results of another analyst's studies of clopidogrel

Method	Added (µg/mL)	Found (µg/mL) (Mean±SD)	% Recovery	% RSD[a]
SWV	5	5.1 ± 0.19	102.0	3.72
	15	14.9 ± 0.24	99.3	1.61
	35	35.1 ± 1.67	100.2	4.76

[a]Six replicate measurements' mean values

Stability

The stability of clopidogrel stock solution was examined over a period of at least 72 hours. Furthermore, clopidogrel standard solutions were stable for 72 hours at 4 and -20 °C refrigeration temperatures as well as ambient temperature. The clopidogrel accuracy is within the acceptable range of 90 to 110% (Table 5). There are no major clopidogrel breakdown products under these circumstances.

Table 5. Clopidogrel's stability at various temperatures (n=6)

Added (µg/mL)	Room temperature 24 h (Mean ± SD)	Room temperature 72 h (Mean ± SD)	Refrigeratory +4 °C, 72 h (Mean ± SD)	Frozen -20 °C, 72 h (Mean ± SD)
15	100.2 ± 1.76	98.3 ± 3.71	101.8 ± 1.37	98.9 ± 2.42
30	98.7 ± 3.14	101.8 ± 3.42	98.7 ± 2.47	100.6 ± 1.49
45	99.5 ± 2.11	101.3 ± 3.52	98.8 ± 1.67	98.4 ± 2.53

Procedure for Pharmaceutical Preparations

Each Plavix and Karum tablet, which contains 75 milligrams of clopidogrel, was precisely weighed and finely powdered. A suitable amount of powder was dissolved in 50 mL of 0.1 M $LiClO_4$/acetonitrile. Then, the final volume was made up to 100 mL in a balloon flask. Whatman filter (paper no 42) was used to filter the tablet solutions after they had been properly diluted in order to provide a final concentration that was within the linearity constraints of the SWV method (Figure 8). The calibration curve was used to determine the drug concentration for clopidogrel (Table 6).

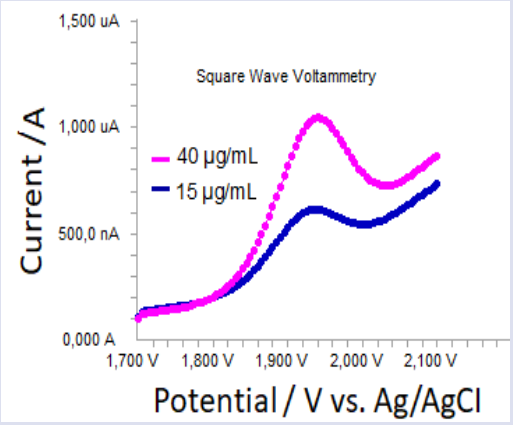

Figure 8. The voltammograms of Plavix tablet containing clopidogrel.

Table 6. The comparison of the presence of clopidogrel in two commercial drugs

Tablet	N	Mean	Standard Deviation	%RSD
Plavix	10	75.1	1.147	1.53
Karum	10	74.6	1.370	1.84

N: The number of analysis (n=6)

Additionally, the official method [20] and the new SWV voltammetric approach were statistically evaluated using the t-test. The computed t-values don't go over the theoretical values at a 95% confidence level (Table 7).

Table 7. Comparison of the methods

Parameters		Official Method
Mean (75 mg per tablet mg)[a]	75.1	74.88
% RSD (Calculated t-value)	0.21[b]	
T theoretical value (p=0.05)		

[a]Each value is the mean of six experiments, [b]NS: not significant

The analytical findings in this investigation showed that the level of active ingredient in the medicine is within the pharmacopoeia's recommended range. The approach can be used as a substitute for the spectrophotometric approach. The developed method was demonstrated to be practical, accurate, and adaptable to drug dose forms. Therefore, developed SWV method can be advised for the routine QC analyses of clopidogrel in pharmaceutical preparations.

Conclusions

In the current work, CV method has been used to examine the electrochemical behavior of clopidogrel in nonaqueous media. Additionally, a quick, accurate, specific, and precise SWV method was developed and validated in the study for the detection of clopidogrel in pharmaceutical formulations. Voltammetry runs for one minute. The method enables the speedy analysis of a large number of samples. As a result, the method can be used to regularly examine clopidogrel in both its formulations and pure form.

Conflicts of interest

The author states that did not has conflict of interests

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