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Determination of Clopidogrel Bisulphate in Plavix Tablet and Human Biological Fluids Utilizing Chemically Modified Carbon Paste Sensor

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Abstract

The fabrication and the performance response characteristics of a novel sensitive, selective, simple, and rapid sensor for the determination of Clopidogrel bisulphate (CLO-H_2SO_4) were described. The sensing modified carbon paste sensor comprised of an ion-pair based on Clopidogrel with silico tungastate (CLO-ST) where this study included: composition, usable pH range, response time and temperature. The sensorex hibiteda wide linear dynamic concentration ranging from $1.00x10^{-7}$-$1.00x10^{-2}$ and the usable pH ranges from 1.2-4.8 with the response time ranging from (5-8 sec) which is much faster compared to liquid ISEs with a detection limit equals 0.34 nM. The selectivity of the sensor (CLO-H_2SO_4) was applied with respect to a many of organic and inorganic cations, amino acids and sugars. The application of the sensor (CLO-H_2SO_4) for its determination was utilized in bulk powder, Plavix Tablet, human (serum-urine) and monitoring Plavix tablet dissolution rates using calibration curve, standard addition, and the potentiometric titration methods. The obtained results were statistically analyzed in both accuracy and precision and were compared using the US pharmacopeia method where there is no significant difference was observed.

Keywords: Clopidogrel bisulphate (CLO-H_2SO_4); Carbon-paste sensor; Potentiometry; Dissolution profile

Introduction

Plavix is a trademark prescription medicine used to treat people who have any of the following:

A heart attack, a Stroke or Recent Stroke, chest pain due to heart problems, poor circulation in their legs (peripheral arterial disease), acute Coronary Syndrome (ACS), established Peripheral Arterial Disease; use with Proton Pump Inhibitors (PPI), clopidogrel reduces the risk of heart attack and stroke in people who have cardiovascular disease [1-4]. Due to vascular diseases such as atherosclerosis, clopidogrel platelet inhibiting activity is an effective drug for reducing the incidence heart attacks, claudicating, or ischemic strokes. Clopidogrel reduces the chance of arterial blockage, by inhibiting platelet aggregation, thus preventing heart attacks and strokes. Clopidogrel bisulfate is methyl (+)-(S)-α-(2-chlorophenyl)-6,7-dihydrothieno[3,2-c] pyridine-5(4H)-acetate sulfate (1:1) with molecular formula $C_{16}H_{16}ClNO_2S.H_2SO_4$ and its molecular mass equals 419.9 as in Figure 1.

The literature survey review refers to different analytical methods such as spectrophotometric methods for determination of CLO [5-9], quantitatively kinetic spectrophotometric for CLO, petaxolol and imidril in its dosage form [10]. Other methods included non-enzymatic and enzymatic chiral inversion of CLO utilizing NMR and HPLC chiral method [11]. The analysis by GC-MS for metabolite carboxylic acid of CLO in serum and plasma [12], reverse phase HPLC with UV detection for estimation of CLO in its dosage form were presented in [13-17], HPTLC [18,19] and HPLC [20-22]. CLO was determined in the presence of its human fluid by mass spectrometry coupled with LC [23,24], and HPLCMS/MS [25]. Also capillary electrophoresis methods were reported [26-28], and voltammetry [29].

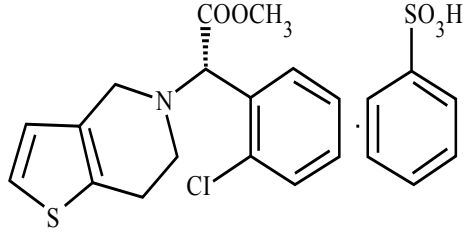

Figure 1: The chemical structure of clopidogrel bisulfate.

The electrochemistry and electro analysis with carbon paste-based sensors, and detectors have been presented and the following two principal conclusions can be made: herein. Firstly: It is the fact that carbon paste still represents one of the most popular sensor materials with almost unlimited applicability in basic research, highly specialized investigations, as well as in practically oriented electro analysis [30,31].

Secondly, the recent turbulences in the area of CPEs, CMCPSs, CP-biosensors, and CP- detectors have shown that carbon paste is also one of the most flexible substrates that obey criteria of the green-chemistry concept; especially, in the area of environmental inorganic analysis. Further adaptations of the already existing methods in terms of improved performance, combination with other techniques, compatibility with analyzers of new generation, or even acceptability given by actual economic and ecologic demands. In this respect, a majority of carbon pastes have great promise as a cheap, easy-to-prepare, and – in the native form – also almost non-toxic material [32].

This work describes the fabrication, construction with sensor potentiometric characterization, and application of a novel clopidogrel-chemically modified carbon paste sensors (CLO-CMCPSs) by the use of clopidogrel silicotungstate (CLO-ST), clopidogrel silicomolybdate

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(CLO-SM), clopidogrel phosphotungstate (CLO-PT), clopidogrel phosphomolybdate (CLO-PM), clopidogrel tetraphenylborate (CLO-TPB) and ion-pairs clopidogrel reineckate (CLO-Rein) with solvent mediator 2-Nitrophenyl phenyl ether (2-NPPE) and compared with sensors previously reported [33,34].

Experimental

Chemicals and materials

All chemicals used for preparation of solutions were of analytical grade. Doubly distilled water was used throughout all the experiments. Clopidogrel bisulfate and its dosage form (Plavix, 75 mg /tablet) were provided by Sanofi Aventis Company Cairo-A.R.E. Tributyl phthalate (TBP), tricresyl phosphate (TCP), graphite powder, dibutyl phthalate(DBP), dioctyl phthalate (DOP), 2-nitrophenyl phenyl ether (2-NPPE), ammonium reineckate (Amm-Rein), sodium tetra phenylborate (NaTPB), silicotungestic acid (STA), silicomolybdic acid (SMA), phospho molybdic acid (PMA), and phospho tungestic acid (PTA),were selective products from Aldrich.

0.5 M chloride solution of each of the following cations: NH_4^+, K^+, Na^+, Zn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Mg^{2+}, Ba^{2+}, Mn^{2+}, Cr^{3+}, and Fe^{3+}solutions(1000 μg ml^{-1}) were obtained from Merck. Glucose anhydrous, lactose monohydrate, maltose, urea, ascorbic acid, aspirin, L-threonine, L-lysine, L-cystine, and L-glycine were obtained from Aldrich. Serum was used within 24 h and provided by VACSERA (Giza, Egypt) while urine samples were obtained from healthy volunteers. Corn oil, sodium hydroxide and hydrochloric acid are from NODCAR.

The electrochemical system

The potentiometric measurements were carried out with a Jenway 3515 digital pH/mV meter. A WTW-packed saturated calomel sensor (SCE) was used as an external reference sensor. The electrochemical system was as follows: CMCPS/test solution//SCE. The dissolution profile was studied using USP XXXII [35] method with the paddle apparatus two [36], which was provided as first choice for in vitro dissolution testing for controlled/modified-release preparations, and more uniform flow profile. The apparatus used for this purpose is model "SR8Plus", CA USA Hanson Research, with number "73-100-116" and the spectrophotometer double beam instrumentUV-1800-2011 Shimadzu (Japan).

Preparation of ion-pair

An ion-pair, clopidogrel silicomolybdate (CLO-SM), clopidogrel phospho molybdate (CLO-PM), clopidogrel silicotungstate (CLO-ST), clopidogrel phosphor tungstate (CLO-PT), clopidogrel tetraphenyl borate (CLO-TPB) and ion-pairs clopidogrel reineckate (CLO-Rein), were prepared according to a previously reported method [37]. The formed precipitates were filtered off, washed thoroughly with distilled water, then dried at room temperature and ground to fine powder.

Sensor preparation

Chemically modified carbon paste sensors were prepared as previously described [37]. The sensor was used directly for potentiometric measurements without preconditioning requirements. A fresh surface of the paste was obtained by squeezing more out. The surplus paste was wiped out and the freshly exposed surface was polished on a paper until the surface showed shiny appearance.

Construction of the calibration graphs

Different compositions were prepared. The percentages of each ion-pair were changed to cover the ranges of 0.5-5% of CLO. Lower concentration than $1.0x10^{-6}$ M was prepared by appropriate dilutions. The measured potential was recorded using the present sensor. Data were plotted as potential versus logarithm of the drug concentration CLO^+ activity and the resulting graph was used for subsequent determination of unknown drug concentration.

Effect of pH

In batch technique measurements the effect of pH of the test solution on the performance of each sensor was investigated by measuring its potential in solutions prepared the concentration range of $1.0x10^{-5}$-$1.0x10^{-3}$ M by serial dilution. The sample solution pH was monitored simultaneously with a conventional glass pH sensor. The emf readings were taken after the potential reached a constant value. The mV-readings were plotted against the pH-values for the different concentrations.

Effect of temperature on the sensor potential

The thermal stability of the sensors with calibration graphs were studied covering the range 25-50°C of different test solution-temperatures. The usable concentration ranges, slope, the response time of the sensor corresponding to each temperature and the standard sensor potentials (E°) were studied. The values of E° were plotted versus (t-25) to the estimation of the thermal coefficients of the sensors. The isothermal coefficient (dE°/dt) of the cell represents the straight line slope obtained by plotting of $E°_{cell}$ versus (t-25) and was calculated for each sensor by the following equation (Andropov's equation):

$$E°_{cell} = E°_{25°C} + (dE°/dt)\ (t-25)$$

By the subtraction of the standard sensors potential of the calomel sensor (241.2, 234.4, 230.88 and 223.57 mV) at different temperatures (25, 35, 40 and 50°C) so the values of the standard potentials of sensors ($E°_{elec}$.) were calculated.

Sensors selectivity

The matched potential method (MPM) [38,39] was applied as the previously reported method [37].

The following equation is used to calculate the selectivity values of: $\log K^{POT}_{CLO,J^{z+}}$

$$K^{POT}_{CLO,J^{z+}} = \frac{(a_A^- - a_{drug})}{a_j}$$

Where: a_A^- is the initial concentration of drug, a_{drug} is the activity of the added drug and a_j is the activity of the added interfering ion producing the same increase in potential.

Potentiometric determination of CLO

The investigated sensors are used to calculate the concentration of the drug in sample solution; where, small portions (0.1 ml) of standard 10^{-2} M CLO solution were added to 50 ml water-containing different concentrations of drug ranging from 10^{-6} to 10^{-4} M or its pharmaceutical dosage form using the following equation:

$$C_X = C_S\left(\frac{V_S}{V_X+V_S}\right)\left[10n(\Delta E/S) - \frac{V_X}{V_S+V_X}\right]$$

Where C_x is the concentration to be determined, V_x is the volume of the original sample solution, V_s and C_s are the volume and concentration of the standard solution added to the sample to be

analyzed, respectively, ΔE is the change in potential after addition of certain volume of standard solution, and S is the slope of the calibration graph.

In the potentiometric titrations of the investigated drug, which containing different concentration from 2.09–41.99 mg CLO-$H_2SO_4$was dissolved in 50 ml by bi-distilled water. Different volumes of this solution (1.0-5.0 mL) were taken and subjected against 0.0025 M STA, 0.0025 SMA, 0.0033 PTA, 0.0033 PMA, 0.01 M (NaTPB and Rein) using the sensor(s). Conventional S-shaped curves with first and second plots were used to determine the end points.

In addition, standard addition method, special amounts of CLO-H_2SO_4 were transferred to the cell covering the range from 5.0×10^{-7} to 1.0×10^{-2} M and the present sensor was used to measure the potential recording. Unknown drug concentration was determined from the graph produced by plotting the logarithm of CLO^+ activity versus the potential.

Determination of CLO in Plavix

For sampling of tablets, five Plavix tablets (75mg/tablet) were powdered together to fine powder. An accurately weighed portion was taken from this powder, was added to 50 ml distilled water and the solution was completed to the mark with distilled water then shaken. The standard additions technique was applied for the potentiometric determination.

Content uniformity assay of Plavix tablets<905> [36]

One tablet of Plavix (75 mg/tablet) was immersed in the measuring flask and adjusted to pH1, for measuring each sensor was immediately putted in the sample solution three times and then washed between each individual measurement with distilled water to reach steady potential. The content uniformity was evaluated from the calibration graph by using the mean potential. For the spectrophotometric measurements by employing UV absorbance λ max 240 nm with the standard solution.

Determination of CLO in biological fluids

In serum: One mL of standard drug solution from $1x10^{-3}$, $1x10^{-4}$ and $1x10^{-5}$ M were added into a three of centrifugation 20 ml stoppered shaking tubes. Each tube containing 9 ml of serum and 0.1 N acetate buffers was added to serum solution drop wise until the suitable pH obtained. The tubes were shaken well for 1 min and 10.0 mL of diethyl ether was added to each tube and centrifuged for 2 min at 1500 rpm. Then, the de-protonated layer was transferred to a 100 mL measuring flask and complete to volume using distilled water. The modified sensor was immersed in conjunction with the reference sensor in these solutions and then washed with water between measurements. The emf produced for each solution was measured by the proposed sensor, and the concentration of CLO-H_2SO_4 was determined from the corresponding sensor calibration and standard addition methods.

In spiked urine: For urine analysis, different quantities of the drug and 5 ml urine were transferred to a 100 ml volumetric flask and left stirred for 5min, completed to the mark with doubly distilled water and a small volume (0.1–2.0 ml) 0.01 M HCl was added to give solutions of pH ranging from 4 to 5 and concentrations from $1.0x10^{-6}$ to $5.0x10^{-4}$ M drug. These solutions were subjected to the standard addition method for drug determination.

Dissolution <711> [36]

One tablet of Plavix (75 mg/tablet) was placed in the vessel of 16 tablet dissolution instruments apparatus 2. In vitro release study the dissolution medium (900 ml of 0.01 M HCl) pH 1.2 was maintained at 37 ± 0.5°C for 2 h. The clopidogrel was kept in hard gelatin capsule so the vessel was rotated at 50 rpm. At appropriate time intervals, the potential values were recorded using the clopidogrel sensor in conjunction with saturated calomel sensor (SCE) reference sensor and the amount of clopidogrel released was calculated from the calibration graph. For the spectrophotometric measurements, 5.0 ml aliquots of the dissolution solution were withdrawn, filtered, diluted with 0.01 M HCl and the concentration of samples was analyzed using UV spectrophotometer (1800, Shimadzu, Japan) and the absorbencies were measured at λmax240 nm. A calibration graph was used for drug release calculation.

Result and Discussion

Composition and performance characteristics of CLO sensors

The paste with no exchangers displayed no measurable response towards clopidogrel (CLO^+) ion. For this purpose, the ion-associates of CLO_4ST, CLO_4SM, CLO_3PT, CLO_3PM, CLO-TPB and CLO-Rein were prepared. The chemical composition of the precipitates was identified and confirmed by elemental analysis (C, H, and N) at the Microanalysis Center, Cairo University, Egypt. The results are shown in Table 1.

While the investigated as modifiers for carbon paste sensors selective to clopidogrel as shown in Table 2. The influence of the binder type and concentration on the characteristics of the studied sensors was investigated by using six binders with different polarities including 2-NPPE, TCP, DOP DBP, TBP and Corn Oil.

Different binder/graphite (w/w) ratios were studied. The sensor with 2-NPPE as a solvent mediator produced the best response, as shown in Figure 2, likely due to better dielectric characteristics of 2-NPPE comparing to other solvents, and the ability of 2-NPPE to extract clopidogrel ions from the aqueous solution to the organic paste phase.

Among the different compositions studied, a paste containing ion-exchanger complex 5.0wt% CLO_4ST, 54.0wt% graphite, and 41.0wt% 2-NPPE exhibited the best response characteristics also the lowest detection limit. Therefore, this composition was used to study various operation parameters of the sensor and the optimum composition for the best sensor was given in Table 3. This sensor was chosen in this study and its electrochemical performance characteristics were systematically evaluated according to IUPAC recommendation [40,41].

Ion-associate	Color	Tentative Formulae		C%	H%	N%
CLO_4-ST	(off-white)	$[C_{16}H_{16}ClNO_2S]_4[SiW_{12}O_{40}]$	Found	23.25	1.96	1.66
			(Calc.)	23.28	1.94	1.69
CLO_4-SM	(buff)	$[C_{16}H_{16}ClNO_2S]_4[SiMo_{12}O_{40}]$	Found	34.25	2.8	2.41
			(Calc.)	34.28	2.86	2.39
CLO_3-PT	(Y. white)	$[C_{16}H_{16}ClNO_2S]_3[PW_{12}O_{40}]$	Found	17.4	1.44	1.25
			(Calc.)	17.45	1.45	1.27
CLO_3-PM	(faint yellow)	$[C_{16}H_{16}ClNO_2S]_3[PMo_{12}O_{40}]$	Found	25.66	2.16	1.86
			(Calc.)	25.68	2.14	1.87
CLO-TPB	(White)	$[C_{16}H_{16}ClNO_2S][C_{24}H_{20}B]$	Found	64.93	4.8	1.87
			(Calc.)	64.94	4.87	1.89
CLO-Rein	(faint pink)	$[C_{16}H_{16}ClNO_2S][Cr(NH_3)_2(SCN)_4]$	Found	26.11	2.97	13.24
			(Calc.)	26	2.98	13.27

Table 1: Elemental analyses of the ion-associates.

Composition % w/w				
Linear range (M)	**Slope(mV/decade)**	**2-NPPE**	**Graphite**	**Ion-exchanger**
CLO_4ST				
1.0 x 10^{-5}-5.0x 10^{-3}	55.5 ± 0.5	45	54	1
5.0 x 10^{-6}-1.0x 10^{-2}	58.9 ± 1.0	43	54	3
1.0 x 10^{-7}-1.0 x10^{-2}	60.0 ± 0.5*	41	54	5
CLO_4SM				
5.0 x 10^{-5}-5.0x 10^{-3}	48.7 ± 1.5	45	54	1
5.0 x10^{-5}-5.0x 10^{-3}	57.9 ± 0.5	43	54	3
8.0 x10^{-5}- 5.0x 10^{-3}	56.3 ± 1.5	41	54	5
CLO_3PT				
5.0 x 10^{-5}-1.0x 10^{-3}	56.7 ± 1.0	45	54	1
5.0 x 10^{-6}-5.0x 10^{-3}	51.5 ± 1.0	43	54	3
1.0 x1 0^{-6}-1.0x 10^{-2}	58.5 ± 0.5	41	54	5
CLO_3PM				
5.0 x 10^{-5}-1.0x 10^{-2}	53.2 ± 1.1	45	54	1
5.0 x 10^{-5}-1.0x 10^{-2}	56.1 ± 1.2	43	54	3
5.0 x 10^{-5}-1.0x 10^{-2}	56.0 ± 0.8	41	54	5
CLOTPB				
1.0 x10^{-5}-1.0x10^{-2}	56.0 ± 1.7	45	54	1
3.0 x10^{-5}-8.0x 10^{-3}	53.3 ± 1.1	43	54	3
5.0 x10^{-6}-1.0x 10^{-2}	57.6 ± 1.5	41	54	5
CLORein				
5.0 x10^{-5}-1.0x10^{-2}	55.0 ± 1.7	45	54	1
5.0 x10^{-5}-8.0x 10^{-3}	52.3 ± 1.1	43	54	3
7.0 x10^{-6}-1.0x 10^{-2}	57.5 ± 1.5	41	54	5
*Selected composition: **5 %CLO-ST with different binders**				
Binders				
1.0 x 10^{-7}-1.0 x10^{-2}	60.0 ± 0.5	41	54	2-NPPE*
5.0 x 10^{-7}-1.0 x10^{-2}	58.5 ± 0.5	41	54	TCP
1.0 x 10^{-6}-5.0 x10^{-3}	56.8 ± 0.5	41	54	DOP
5.0 x 10^{-6}-5.0 x10^{-3}	53.2 ± 0.5	41	54	DBP
1.0 x 10^{-7}-5.0 x10^{-3}	52.0 ± 0.5	41	54	TBP
5.0 x 10^{-8}-1.0 x10^{-4}	57.0 ± 0.5	41	54	Corn Oil (Afia)

*Selected Binder

Table 2: Composition and slope of calibration curves for different clopidogrel carbon paste sensors at 25.0 ± 0.1°C.

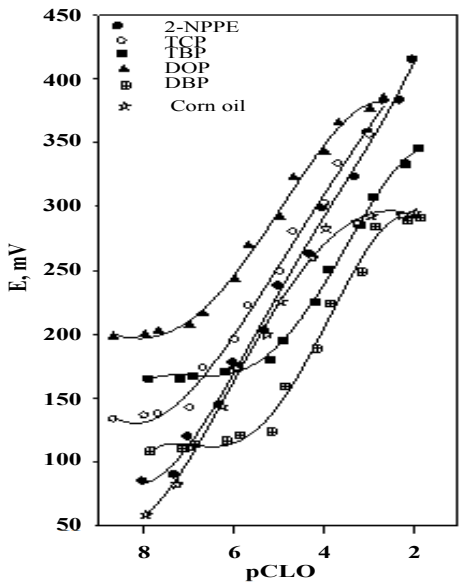

Figure 2: Effect of different plasticizers on the responses of 5% CLO-ST.

Sensor-composition (W/W %)	.(5% CLO_4ST,54% graphite, 41% 2-NPPE)
Slope (mV/decade)	60.0 ± 0.5
Correlation coefficient (r)	0.999
Limit of detection (M)	3.5 x 10-8
Linear range (M)	1.0 x 10-7-1.0 x 10-2
Working pH range	1.2-4.8
Response time (s)	≤ 8
Accuracy (%)	99.87 ± 0.177
Standard deviation (%)	0.326
Robustness	99.79 ± 0.23
Ruggedness	99.83 ± 0.26

Table 3: Response characteristics of CLO-CMCPS at 95% confidence intervals at 25.0 ± 0.1°C.

Reproducibility of the sensor

Compared to other types of modified sensors, CMCPSs have a main advantage in that the surface coverage of a modifier can be adjusted by means of a suitable ratio of carbon paste-to- modifier. The repeatability of the potential reading of the CLO-ST/CMCP sensor was examined by subsequent measurements in 1.0 x 10^{-3} M CLO-H_2SO_4 solution immediately after measuring the first set of solution at 1.0 x 10^{-4} M CLO-H_2SO_4. The standard deviation for 5 replicate measurements of emf was found to be 0.473 in 1x10^{-4} M solution and 0.816in 1x10^{-3} solution.

The slope of the calibration graph obtained by this sensor was found to decrease slightly after several times of use, which may be attributed to surface contaminations. Accordingly, a paste of optimum composition and suitable weight (2.0 g) can be used for several months without any deterioration or change in the response of the sensor. The slope of the calibration graph was found to decrease slightly from 60.0to 55.5 mV/decade after three times of use. This decrease may be attributed to memory effect due to the surface contamination.

It was noticed that the slope of the calibration graph obtained by CLO-ST/CMCPS was nearly constant by polishing for any time taken days and then starts to decrease gradually without polishing so it consider as a new sensor with every one polishing to the sensor. After any period of time a new section from the master paste was found to function very properly.

Dynamic response time

The response time of the sensor is defined as the time between addition of the analyte to the sample solution and the time when a limiting potential has been reached. The dynamic response time [42] of sensor was tested by measuring the time required to achieve a steady-state potential (within ± 1 mV) after successive immersions of the sensor in a series of drug solutions, each having a 10-fold increase in concentration from 1.0 x 10^{-7} to 1.0 x 10^{-2} M. In this study, practical response time was recorded by increasing CLO concentration by up to 10-fold. The sensor yielded steady potential within 10-12 sec. This is most probably due to the fast exchange kinetics of association–dissociation of clopidogrel ion with the ionophores at the solution–paste interface. The potential–time plot for the response of the sensor CLO-ST is shown in Figure 3.

Effect of pH

The potential pH profile obtained indicates that the responses of the sensors are fairly constant over the pH range 1.2–4.8 in this range the sensor can be safely used for CLO determination. For quantitative measurements with ion selective sensors, studies were carried out to reach the optimum experimental conditions. Therefore, the pH range from 1.2 to 4.8 was assumed to be the working pH range of the sensors.

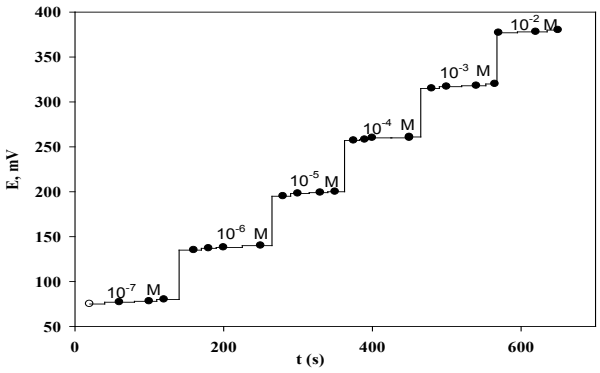

Figure 3: Potential-time plot for response of the CLO-ST/CMCPS.

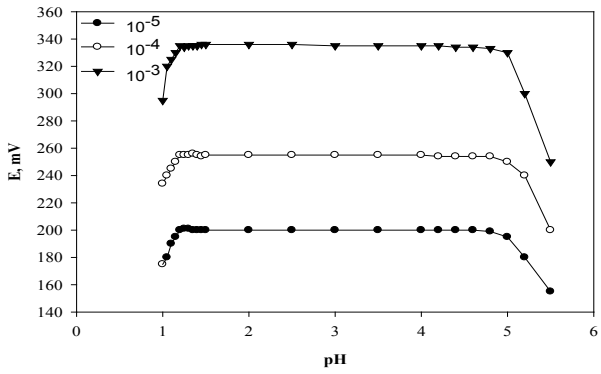

Figure 4: Effect of pH on the response of CLO-ST/CMCPS.

It can be seen from Figure 4 that at pH values lower than the previously mentioned pH ranges, the potential readings decrease which can be related to interference of hydronium ion while at pH values higher than pH 4.8, the potential readings decrease gradually due to the formation of free base of the drug and decrease of the protonated species in the test solutions as shown in Figure 4.

Effect of temperature

Thermal stability of the sensors: To study the thermal stability of the sensors, calibration graphs (emf, mV vs. p Drug) were constructed at different test solution temperatures covering the range 25-50°C. The results indicate that the slopes of the calibration graphs still in the Nernstian range in spite of the increase of the temperature of the test solutions up to 50°C as shown in Figure 5a.

Determination of the thermal coefficient of the sensors: The potential of ion-selective sensors is usually affected by the temperature of the test solution. A thermally stable sensor is characterized by low thermal temperature coefficient. This means the successful applicability of the sensor over a wide range of temperature. To calculate the thermal coefficient of the cell $(dE°/dt)_{cell}$, the standard cell potentials, $E°_{cell}$, were determined at different temperatures from the respective calibration plots as the intercept of these plots at p Drug = 0. Knowing that $E°_{cell}$ is related to (dE°/dt) by the equation:

$$E°_{cell} = E°_{25}°C + (dE°/dt)\ (t\text{-}25)\ °C$$

Plot of $E°_{cell}$ versus (t-25) °C produced a straight line; the slope of this line is taken as the thermal coefficient of the cell, as shown in Figure5b. The value of the standard potentials of sensor (E°elec.) was calculated after the subtraction of the standard sensor potential of the calomel sensor at different temperatures (the values are 241.2, 234.4, 230.88 and 223.57 mV at 25, 35, 40 and 50°C, respectively).Plots of ($E°_{elec}$.) versus (t-25) °C gave a straight line. The slope of the line was taken as the thermal coefficient of the sensor.

The isothermal coefficient (dE_{elec}.,/dt) of the sensor was calculated and found to be ~0.0001 $V°C^{-1}$ and (dE_{cell}/dt) equals ~0.0005 $V°C^{-1}$. These values indicate a fairly high thermal stability of the sensor within the temperature range investigated and show no large deviation from the theoretical Nernstian behavior.

Selectivity of the sensor

The selectivity coefficients presented in Table 4 indicate that, CLO-ST sensor is highly selective to clopidogrel cation. Most inorganic cations do not interfere because of the difference in their mobility and permeability as compared to clopidogrelcation. In the case of sugars and amino acids the high selectivity is related to the difference in polarity and lipophillic nature of their molecules relative to clopidogrel cation.

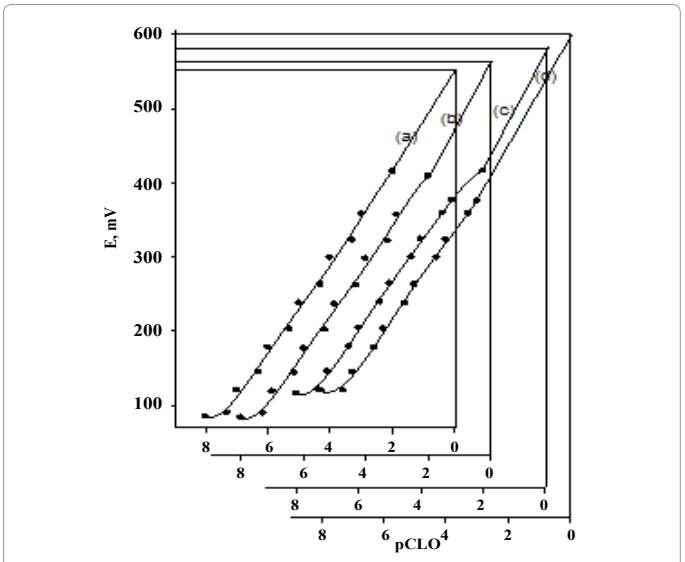

Figure 5a: E Calibration graphs for CLO-ST/CMCPS at the test solution temperatures 25°C(a), 35°C(b), 40°C(c) and 50°C(d).

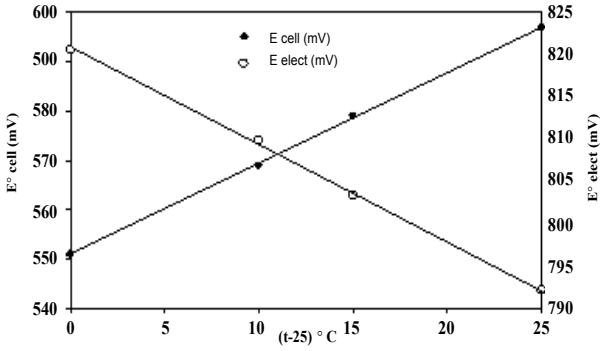

Figure 5b: Variation of standard electrode potential and standard potential of the cell containing CLO-ST/CMCPS with changes of test solution temperatures.

Interferent[b]	MPM[a]	Interferent[b]	MPM[a]
K^+	2.88	Cr^{3+}	3.33
Na^+	2.95	Maltose	3.52
NH_4^+	2.65	Glucose	3.50
Ba^{2+}	3.21	Lactose	3.54
Cu^{2+}	3.20	Urea	3.23
Co^{2+}	3.11	Ascorbic acid	3.30
Ni^{2+}	3.19	Aspirin	1.15
Mn^{2+}	2.56	L-Lysine	3.34
Mg^{2+}	2.89	L-cystine	3.20
Zn^{2+}	2.67	L-Glycine	3.19
Fe^{3+}	3.27	L-Theronine	3.44

[a]Each value is the average of three determinations.
[b]All interferents ions are in the form of 0.5 M chloride solution

Table 4: Selectivity coefficient values for $\log K^{POT}_{CLO,J^{z+}}$ CLO-ST/CMCPE.

I- Standard addition methoda			III- Potentiometric titrations				
			Pure solutions			Plavix tablets	
Taken (mg)	Recovery (%)	R.S.D.%	Taken (mg)	Recovery (%)	R.S.D.%	Recovery (%)	R.S.D.%
Pure solutions			**STA as titrant**				
2.1	99.1	0.63	6.3	99.6	0.5	98.5	0.65
4.2	99.3	0.55	8.4	99.1	0.77	98.1	1.41
6.3	99	0.81	12.6	99	0.89	98	1.08
8.4	98.2	0.64	21	98.2	0.99	97.3	0.97
Plavix tablets			**PTA as titrant**				
2.1	99	0.22	4.2	99.3	0.77	99	1.05
4.2	98.9	0.48	12.6	98.7	0.59	98.6	1.04
6.3	98	0.51	21	98	0.47	98.5	1.18
II- Calibration curve method			29.4	98	0.68	98.2	0.96
6.3	99.3		42	97.6	0.78	97	1.44
8.4	98.6		**SMA as titrant**				
12.6	98		2.1	99.7	0.44	99	0.85
Plavix tablets			4.2	99.3	0.67	99.4	0.64
2.1	98.4		6.3	99	0.78	98.6	0.84
4.2	98.5		8.4	98.5	0.51	98	0.77
6.3	98.7		12.6	98.3	0.73	98.2	1.11
Spiked urine[a]			29.4	98	0.8	97	1.23
2.1	99	0.21	**PMA as titrant**				
4.2	98.6	0.24	4.2	99.8	0.16	99.5	0.84
6.3	98.3	0.43	6.3	99.4	0.77	99.3	0.26
Spiked human serum[a]			8.4	99	0.78	99.1	0.65
2.1	98.3	0.67	12.6	98.5	0.58	98.5	0.91
4.2	98	0.48	29.4	98	0.88	98	0.38
6.3	97.6	1.09	42	98.3	0.3	97.8	0.69

Table 5: Determination of clopidogrel bisulfate in bulk solutions, tablet, urine and serum applying the standard addition method, calibration curve method and potentiometric titrations using CLO-ST/CMCPS.

Quantification of CLO

In order to assess the validity of the proposed sensor, the analytical applications involve determination of the drug in its bulk powder, pharmaceutical preparation (Plavix 75 mg) and biological fluids (serum and urine) was applied. Applying the standard addition method [40], the percentage recovery for determinations of CLO in pure solution, Plavix Tablets and in spiked urine & human serum ranged from 98.2-99.3%, 98.0-99.0, 98.3-99.0 and 97.6-98.3 respectively (Table 5).

While in Calibration curve method the percentage recovery for determinations of CLO in pure solution and Plavix Tablets are ranged from 98.0-99.4 and 98.3-98.7 respectively. The potentiometric titration technique usually offers the advantage of high accuracy and precision, a further advantage is that the potential break at the titration end-point must be well defined. The titration process was carried out manually in aqueous solution containing 4.20-42.0 mg CLO with average recoveries of 98.2-99.6% using NaTPB as titrant, 97.3-98.5% in Plavix tablets.

On using PTA as titrant, 97.6-99.3%, 97.0-99.0%, on using SMA as titrant, 98.0-99.7%, 97.0-99.4, and on using PMA as titrant, 98.0-99.8%, 97.8-99.5% for pure solution and Plavix tablets respectively (Figure 6). The results applying the potentiometric titration method (Table 5). The results obtained were compared with those of official method [35]. No significant difference between two methods was observed with respect to accuracy and precision (Table 6).

Validation of the proposed method

Linearity and detection limit (LOD): Under the optimal experimental CMCPS conditions, a linear relationship exists between the sensor potential /mV and the logarithm of corresponding concentration of the investigated drug, the value of LOD was indicating that the proposed method is sensitive for detection of very small concentrations of CLO reach to 0.35nM. The correlation coefficient (r) and other statistical parameters were listed in Table 3.

Accuracy: The accuracy of the proposed CMCPS method was investigated by the determination of CLO in its pharmaceutical preparations without interfering from the co formulated adjuvant as indicated by the mean recovery value of 99.87 ± 0.177mV/decad for the investigated sensor.

Precision: The precision of the CMCPS method measured as percentage relative standard deviation (% RDS) was tested by repeating the proposed CMCPS method for analysis of the investigated CLO in intra-day (within the day) and inter-day(consecutive days) to five replicates. The obtained % RSD values were 0.379%, o.466% for the sensor. The% RSD values are less than 2%, indicating good precision.

Analytical applications

The standard addition method was proved to be successful for the determination of clopidogrel in its bulk solutions, Plavix tablet (75 mg/tablet)and biological fluids (human serum and urine) using its prepared chemically modified carbon paste sensor.

Determination of Plavix tablet (75 mg/tablet): In order to assess

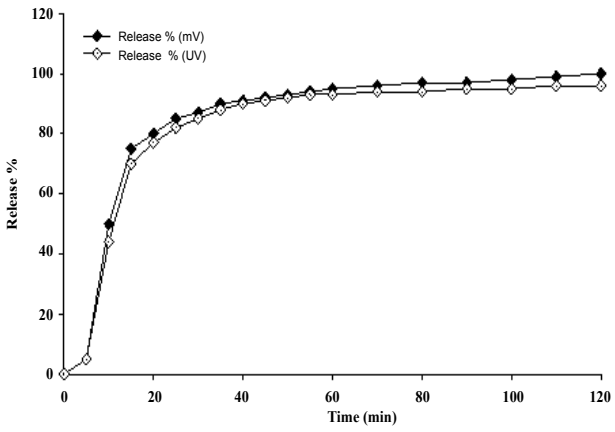

Figure 6: Dissolution profiles of 75 mg clopidogrel bisulfate tablet obtained by potentiometric: 5.0% CLO-CMCPE, and spectrophotometric measurements at 240 nm.

Pure solution				Tablet		
Method	X ± S.E.	Relative error (%)	$F^{3.3}$ value (9.28)	X ± S.E.	Relative error (%)	$F^{3.3}$ value (9.28)
Official method[35]	98.0 ± 0.50	0.851		97.0 ± 0.6	0.90	
Method (I)	99.2 ± 0.11	0.891	0.785	98.6 ± 0.112	0.621	0.566
Method (II)	99.5 ± 0.46	0.624	0.748	98.9 ± 0.013	0.651	0.901
Method (III)	99.7 ± 0.06	0.339	0.806	99.5 ± 0.372	0.917	0.739
Spiked urine				**Spiked human serum**		
Official method[35]	98.0 ± 0.44	0.822		98.1 ± 0.2	0.911	
Method (I)	98.7 ± 0.73	0.621	0.625	98.5 ± 0.035	0.481	0.577

Method (I): The standard addition method
Method (II): The Calibration curve method
Method (III): The Potentiometric titrations method
X ± S.E: Recovery ± standard error
F- tabulated is 6.39 at 95.0% confidence limit
t- tabulated at 99.0% confidence limit and 6 degrees of freedom

Table 6: Statistical treatment of data obtained for the determination of CLOusing the CLO-ST/CMCPE.

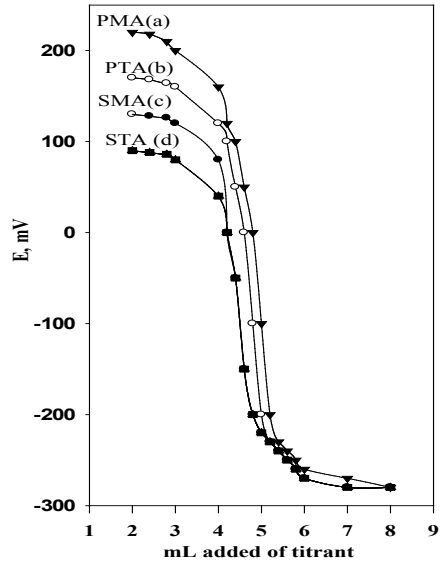

Figure 7: Potentiometric titrations of 21.0 mg CLO-H_2SO_4 with PMA (a), PTA (b), SMA (c) and STA (g) as titrants using CLO-ST/CMCPS.

the validity of the proposed sensor, the standard additions method [40], calibration curve method and the potentiometric titration method (Table 5 and Figure7) show the determination of CLO in its bulk solutions and tablet. The results also prove the applicability of the three methods for the determination of CLO in the pharmaceutical formulation.

Determination of CLO in (Human serum and urine): The proposed CMCPS method was successfully applied to determine CLO in biological fluids and the results obtained were summarized in Table 5. The determination of CLO in spiked human serum shows that a wide concentration range of the drug can be determined by the investigated sensor with high precision and accuracy. In urine samples the standard addition technique was applied to overcome the matrix effects in these samples. Also, the response times of the proposed sensors are instant (within 15 sec), so the sensors are rapidly transferred back and forth between the biological samples and the bi-distilled water between measurements to protect the sensing component from adhering to the surface of some matrix components. It is concluded that the proposed sensors can be successfully applied to in vitro studies and for clinical use. This confirms that the sensitivity and limit of quantification (LOQ) are adequate for determination of clopidogrel bisulfate in pharmacokinetic studies.

Potentiometric monitoring of Plavix tablet dissolution [35,36]: The dissolution test was operated at 50 rpm in 900 ml $1.0x10^{-2}$ M hydrochloric acid(simulated duodenum fluid), and the use of potentiometric clopidogrel sensor. The simulated duodenum fluid was kept at 37.0 ± 0.5°C. There are no degradation products in the vitro test. The compression recipients do not interfere. Taking into account the S-shape of the dissolution curve obtained (Figure 7). It shows that clopidogrel releases immediately after capsule was ruptured. More than 75% drug was released within 15 min and complete dissolution was achieved in 120 min.

The potentiometric method, the potential values were continuously recorded at 1-min time intervals and compared with a calibration graph. For the UV spectrophotometric assay, fixed volumes of the dissolution medium were withdrawn, diluted with 0.01 M HCl, measured at λ max 240 nm and compared with a calibration graph. Figure 6 shows the dissolution profiles of clopidogrel tablet using both measurement techniques. The results obtained by spectrophotometric and potentiometry are almost identical. The use of the potentiometric method sensor, however, has the advantage of in situ monitoring.

Robustness and ruggedness: The robustness method of the CLO-CMCPS was examined by changed the aqueous solution to acetate buffer pH 4 ± 0.5 and the percentage result was 99.79 ± 0.23 mV/decad for the CLO-ST.

This result was closely in agreement with those obtained from standard drug solution, (Table 1).While the ruggedness or the reproducibility was checked by using another model of pH-meter (Jenway, 3505) was indicated by the results obtained as percentage was 99.83 ± 0.26 mV/decad for the same sensor (Table 1).

Content uniformity assay of Plavix tablets: The proposed ISE method described good accuracy and precision for the quality control tests, the content uniformity assay showed that accurate and reproducible results so the sensor can be employed for quantification of clopidogrel and the recovery of CLO-H_2SO_4 is almost quantitative.

Statistical treatment of results: The results obtained from the potentiometric determination of the drug in these real samples are given in Table 5. The results of the recoveries of CLO applying the standard additions method, calibration curve method and the potentiometric titration were evaluated statistically and compared with the values obtained with the pharmacopeia method by applying the F-tests [43]. The values obtained (Table 6) show that the present methods have a precision comparable to that of the pharmacopeia method. However, the proposed methods are more practical regarding time of analysis, consumption of solvents and sample pretreatment requirements for spectrophotometric or chromatographic analysis of clopidogrel bisulfate.

Comparison of the clopidogrel selective sensors: The performance characteristics of the proposed sensor and those of some reported ISE method are presented in Table 7 for comparison. It is clear that the proposed sensor CMCPS is comparable with most of the reported sensors with regard to working concentration range, response time

Reagent/method	Linear range µM/ L	LOD µM/ L	r^2	R%	RSD%	λmax nm	Ref.
Spectrophotometric							
0.1N HCl	5.0 - 25.0	10	<1	99.77	--	211.3	[6]
Chemometric	50-100	0-20	0.998	95-105	1.18	220-250	[9]
RP-HPLC	1.5-7.5	0.05	0.999	99.3-100.6	0.067	240	[13]
RP-HPLC	0.2–3.5	0.079		97.3-99.9	1.36	235	[14]
RP-HPLC	5-30	0.66	0.999	97.2-99.6	< 2%	235	[16]
HPTLC	400-1400 ng/spot	--	0.998	100.16 ± 0.6	< 2%	235	[18]
HPTLC	300-600 ng/spot	8.8 ng	0.9866	98.5 - 101.2	<1.5%	220	[19]
HPLC-UV	0.005- 5.0 µg	2.0 ng	0.999	>99%	<6.38	220	[22]
LC/MS/MS	10 - 10,000 pg	10 pg	> 0.99	98.4-103.5	< 2%	--	[23]
LC-MS/MS	0.5 to 250 ng	--	0.998	85 - 105	< 2%	--	[24]
Capillary (CZE)	0.4-300	0.13	0.998	>99%	< 2%	--	[26]
Cyclic voltammetry	0.08 -1.0 mM	0.04	0.998	99.2-101.6	< 2%	--	[29]
GC-MS	5-250 ng	2 ng	0.999	92 - 114	< 2%	--	[12]
ISS	**Ref [33]**		**Ref [34] [C.S]**			**[C.S]**	
	TpClPB- (PVC)		**(PMA)**	**(ARS)**	**(PTA)**	**CMCPS**	
			(DBP)			**(2-NPPE)**	
Parameter	**o-NPOE**	**DOP**	**Plastic**	**Coated-Ag**	**Coated-graphite**	**CLO-ST**	
Slope (mV/decade)	61.7	59.3	55.97	57.57	58.03 ± 0.150	60.0 ± 0.5	
Correlation coefficient (*r*)	0.9874	0.9993	0.9998	0.9999	0.9999	0.9999	
Linear range (M)	--	--	1×10^{-7}-1×10^{-2}			1×10^{-7}-1×10^{-2}	
LOD (M)	1.0×10^{-5}	5.01×10^{-8}	4.1×10^{-8}	5.0×10^{-8}	3.5×10^{-8}		
Working pH range	1.5 - 4.0	1.2-4.6	1.2-4.8				
Response time (s)			20	25	15	≤ 8	
Life span/days	77	84	25	30	40	Fresh surface	
Accuracy (%)	--	--	99.09 ± 0.6	99.72 ± 0.24	99.28 ± 0.857	99.87 ± 0.177	
Standard deviation	--	--	0.460	0.227	0.150	0.326	
Robustness			99.48 ± 0.537	99.36 ± 0.337	99.51 ± 0.445	99.79 ± 0.23	
Ruggedness			99.51 ± 0.430	99.26 ± 0.650	98.77 ± 0.199	99.83 ± 0.26	

r^2: Correlation coefficient R%: Mean recovery% C.S: Current Study

Table 7: Comparison between some of the published and the suggested methods for determination of CLO-ion.

and low detection limit. Overall evaluation indicates this sensor is more useful in such applications.

Conclusion

The proposed potentiometric methods based on the construction of different types of selective sensors with ion exchangers might be useful analytical characteristics for the determination of CLO in its bulk solutions, pharmaceutical dosage form and biological fluids. The good recoveries and low relative standard deviations obtained reflect the high accuracy and precision of the proposed method. Moreover, the method is simple, easy to operate, high sensitivity, reasonable selectivity, fast static response, long term stability and applicability over a wide concentration range with minimal sample pretreatment and inexpensive making it an excellent tool for the routine determination of CLO in quality control laboratories. The sensor developed is superior as compared with the clopidogrel selective sensor described in the literature [33,34].

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References

1. Budavari S (2011) The Merck Index. 13th Edtn, Merck & Co, USA.
2. Henein HW (2006) ATLAS 2 everything about drugs from A to Z. Nobar publisher, USA.
3. Gomes Y, Adams E, Hoogmartens (2004) Analysis of purity in 19 drug product tablets containing clopidogrl: 18 copies versus the original brand. J Pharma Biomed Anal 34: 341-348.
4. Taubert D, Kastrati A, Gorchakova O, Lazar A, Von Beckerath N, et al., (2004) Pharmacokinetics of clopidogrel after administration of a high loading dose. Thromb Haemost 92: 311-316.
5. Nayana P, Yogita S, Mahajan NS, Pore YV, Kuchekar BS, et al., (2009) Spectrophotometric estimation of clopidogrel bisulphate in pharmaceutical dosage forms. Int J Chem Sci 7: 216-218.
6. Game MD, Gabhane KB, Sakarkar DM (2010) Quantitative analysis of clopidogrel bisulphate and aspirin by first derivative spectrophotometric method in tablets. Ind J Phama Sci 72: 825-828.
7. Gurav S, Tembare R, Salunkhe V, Devprakash A (2011) Spectrophotometric determination of clopidogrel bisulphate in pharmaceutical formulations. J Pharma Tech Res 1: 258-263.
8. Cholke PB, Ahmed R, Chemate SZ, Jadhave KR (2012) Development and validation of spectrophotometric method for clopidogrel bisulphate in pure and in film coated tablet dosage form. Arch Appli Sci Res 4: 59-64.
9. Rujput SJ, George RK, Ruikar DB (2008) Chemometric simultaneous estimation of clopidogrel bisulphate and aspirin from combined dosage. Ind J Pharma Sci 70: 450-454.
10. El Yazbi F, Mahrous M, Hammad H, Sonji G, Sonji N (2010) Kinetic spectrophotometric determination of betaxolol, clopidogrel and imidapril in pharmaceutical. Curr Anal Techn 6: 228-236.
11. Reist M, Roy M, Montseny JP, Mayer JM, Carrupt PA, et al., (2000) Very

slow chiral inversion of clopidogrel in rats: a pharmaceutical and mechanistic investigation. Drug Metab Dispos 28: 1405-1410.

12. Lagorce P, Pere Y, Ortiz J, Necciari J, Bressolle F (1998) Assay method for the carboxylic acid in human plasma by gas chromatography mass spectrometry. J Chromatogr B Biomed Sci Appl 720: 107-117.

13. Shrivastava PK, Basniwal PK, Jain D, Shrivastava SK (2008) Concurrent estimation of clopidogrel bisulphate and aspirin in tablets by validated RP-HPLC method. Indian J Pharm Sci 70: 667-669.

14. Alarfaj NA (2012) Stability-indicating liquid chromatography for determination of clopidogrel bisulphate in tablets: Application to content uniformity testing. J Saud Chem Soc 16: 23-30.

15. Panda SS (2010) Ion-pairing RP-HPLC method for simultaneous determination of aspirin and clopidogrel bisulphate in tablet and capsule dosage form. Int J Pharma Tech Res 22: 269-273.

16. Londhe SV, Deshmukh RS, Mulgund SV, Jain KS (2011) Development and validation of a reversed-phase HPLC method for simultaneous determination of aspirin, atrovastatin calcium and clopidogrel bisulphate in capsules. Ind Pharma Sci 73: 23-29.

17. Sultana N, Ali AK, Arayne MS, Nawaz M (2011) Simultaneous determination of clopidogrel and aspirin by RP-HPLC from bulk material and dosage formulations using multivariate calibration technique. J Chromatogr Sci 49: 165-169.

18. Petal RB, Shankar MB, Petal MR, Bhatt KK (2008) Simultaneous estimation of acetylsalicylic and clopidogrel bisulphate in pure powder and tablet formulations by high performance column liquid chromatography and high-performance thin-layer chromatography. J AOAC Int 91: 750-755.

19. Sinha PK, Damel MC, Bothara KG (2009) A validated stability indicating HPTLC method for determination of aspirin and clopidogrel bisulphate in combined dosage form. J Anal Chem 4: 152-160.

20. Saber AL, Elmosallamy MA, Amin AA, Killa H (2007) Liquid chromatographic and potentiometric methods for determination of clopidogrel. J Food Drug Anal 16:11-18.

21. Shu-qin L, Jian-wen L, Ji H, Xiao-fan S, Jin S, et al., (2008) Determination of the content of the compound clopidogrel bisulphate tablets by HPLC. J Shen Pharma Univ 25: 983-986.

22. Javed MK, Iqbal Z, Khan A, Shah Y, Ahmed L (2011) Development and validation of HPLC-UV method for the determination of clopidogrel in pharmaceutical dosage form and human plasma. J Liq Chromatogr Rela Techno 34: 2118-2129.

23. Shin BS, Yoo SD (2007) Determination of clopidogrel in human plasma by liquid chromatography/tandem mass spectrometry: application to clinical pharmacokinetic study. Biomed Chromatogr 21: 883-889.

24. 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.

25. Silvestro L, Gheorghe M, Iorachescu A, Ciuca V, Tudoroniu Aet al., (2011) Development and validation of an HPLC-MS/MS method to quantify clopidogrel acyl glucuronide, clopidogrel acid metabolite, and clopidogrel in plasma samples avoiding analyte back-conversion. Anal Bional Chem 401: 1023-1034.

26. Fayed AS, Weshahy SA, Shehata MA, Hassan NY, Jochen P, et al., (2009) Separation and determination of clopidogrl and its impurities by capillary electrophoresis. J Pharma Biomed Anal 49: 193-200.

27. Karazniewicz M, Glowka F, Oszkinis G (2010) Capillary zone electrophoresis method for determination of (+)-S clopidogrel carboxylic acid metabolite in human plasma and urine designed for biopharmaceutic studies. J Chromatogr B 878: 1013-1018.

28. Serra H, Bronze M, Simplicio B (2010) Simultaneous determination of clopidogrel and its carboxylic acid metabolite by capillary electrophoresis. J Chromtogr B 878: 1480-1486.

29. Dermis S, Aydogan E (2010) Electrochemical study of the antiplatelet agent clopidogrel and its determination using differential pulse voltammetry in bulk form and pharmaceutical preparations with a glassy carbon sensor. Pharmazi 65: 175-181.

30. Moghaddam HM, Malakootian M, Beitollah H, Biparva P (2014) Nanostructured Base Electrochemical Sensor for Determination of Sulfite. Int J Electrochem Sci 9: 327-341.

31. Švancara I, Vytřas K, Kalcher K, Walcarius A, Wang J (2009) Carbon Paste Sensors in Facts, Numbers, and Notes: A Review on the Occasion of the 50-Years Jubilee of Carbon Paste in Electrochemistry and Electroanalysis. Electroanalysis 21: 7-28.

32. Švancara I, Walcarius A, Kalcher K, Vytřas K (2009) Carbon paste sensors in the new millennium. Cent Eur J Chem 7: 598-656.

33. Saber AL, Elmosallamy MA, Amin AA, Killa HM (2008) Liquid Chromatographic and Potentiometric Methods for Determinations of Clopidogrel. J of Food and Drug Analysis 16: 11-18.

34. Bin-Ibrahim SF, Alarfaj NA, Aly FA (2012) Determination of Clopidogrel bisulphate Using Ion-Selective Sensors in Bulk, Pharmaceutical Formulation and in Biological Fluids. J of American Science 8: 276-283.

35. United States Pharmacopeia Convention (2009) USP 32 NF 27: United States Pharmacopeia [and] National Formulary. United States Pharmacopeia Convention, USA.

36. Liddell MR, Deng G, Hauck WW, Brown WE, Wahab ZS, et al., (2007) Evaluation of Glass Dissolution Vessel Dimensions and Irregularities. Dissolution Technologies 14: 28-33.

37. Khorshid AF, Issa YM (2014) Modified carbon paste sensor for the potentiometric determination of neostigmine bromide in pharmaceutical formulations, human plasma and urine. Biosensors and Bioelectronics 51: 143-149.

38. Umezawa Y, Buhlmann P, Umezawa K, Tohda K, Amemiya S (2000) Potentiometric selectivity coefficients of ion-selective electrodes. Part I. Inorganic cations (Technical report). Pure Appl Chem 72: 1851-2082.

39. Umezawa Y, Umezawa K, Sato H (1995) Selectivity coefficients for ion-selective sensors: Recommended methods for reporting KA,B pot values (Technical report). Pure Appl Chem 67: 507-511.

40. Recommendations (1975) Recommendations for nomenclature of ion-selective elctrodes. Pure Appl Chem 48: 127-132.

41. Linder E, Umezawa Y (2008) Performance evaluation criteria for preparation and measurement of macro- and micro fabricated ion-selective sensors. Pure Appl Chem 80: 85-104.

42. Skoog DA, Holler FJ, Neiman TA (1998) Principles of Instrumental Analysis. 5th edtn. Saunders College Publishers, USA.

43. Miller JC, Miller JN (1992) Statistics for Analytical Chemistry. 2nd edtn, Ellis Horwood, England.