New Analysis 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$_2$SO$_4$) were described. The sensing modified carbon paste sensor comprised of an ion-pair based on clopidogrel with silicotungstate (CLO-ST) where this study included: composition, usable pH range, response time and temperature. The sensor exhibited a wide linear dynamic concentration ranging from 1.00×10$^{-2}$-1.00×10$^{-7}$ and the usable pH ranges from 1.2-4.8 with the response time ranging from (5-8 s) which is much faster compared to liquid ISEs with a detection limit equals 0.34 nM. The selectivity of the sensor (CLO-H$_2$SO$_4$) was applied with respect to a many of organic and inorganic cations, amino acids and sugars. The application of the sensor was utilized in bulk powder, Plavix Tablet, human (serum-urine) and monitoring Plavix tablet dissolution rates. 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$_2$SO$_4$); Carbon-paste Sensor; Potentiometry; Dissolution rate

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. Clopidogrel reduces the risk of heart attack and stroke in people who have cardiovascular disease [1-4]. 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)-acetic acid sulfate (1:1) with molecular formula C$_{16}$H$_{16}$ClNO$_2$S.H$_2$SO$_4$ and its molecular mass equals 419.9 as shown 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, petaxol 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 HPLC-MS/MS [25]. Also capillary electrophoresis methods were reported [26-28], and voltammetry [29]. No methods are found in the literature for determination of CLO by chemical modified carbon paste (CMCPs).

The electrochemistry and electroanalysis with carbon paste-based sensors is 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 electroanalysis [30-32].

This work describes the fabrication, construction with sensor potentiometric characterization, and application of a novel clopidogrel-chemically modified carbon paste sensors (CLO-CMCPs). Using the following ion-pairs; clopidogrel silicotungstate (CLO-ST), clopidogrel siliconolydate (CLO-SM), clopidogrel phosphotungstate (CLO-PT), clopidogrel phosphomolybdate (CLO-PM), clopidogrel tetrophenylborate (CLO-TPB) and 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 bisulphate and its dosage form (Plavix, 75 mg/tablet) were provided by Sanofi Aventis Company Cairo-A.R.E.
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 II [36]. The apparatus used for this purpose is model “SR8Plus,” CA USA Hanson Research, with number “73-100-116”) and the spectrophotometer double beam instrument UV-1800-2011 Shimadzu (Japan).

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 cover the ranges of 0.5-5% of CLO were prepared. The measured potential was recorded using the present sensor. Data were plotted as potential versus logarithm of the drug and the interferents. The selectivity values of \( K_{\text{CLO,J}}^{\text{pot}} \) are calculated using the following equation:

\[
\log K_{\text{CLO,J}}^{\text{pot}} = \frac{(a_J - a_{\text{drug}})}{a_J}
\]

Where: \( a_J \) is the initial concentration of drug, \( a_{\text{drug}} \) is the activity of the added drug and \( a_J \) is the activity of the added interfering ion producing the same increase in potential.

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 \( K_{\text{CLO,J}}^{\text{pot}} \):

\[
\log K_{\text{CLO,J}}^{\text{pot}} = \frac{E_1 - E_2 + \log [\text{Drug}] - \log [J^{\text{pot}}]^{1/2}}{S}
\]

Where: \( E_1 \) and \( E_2 \) are the sensor potentials of 10\(^{-3}\) M solution of each of the CLO drug and interfering cation, \( J^{\text{pot}} \), respectively and \( S \) is the slope of the calibration graph.

Potentiometric determination of CLO

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\(^{-4}\) to 10\(^{-3}\) M or its pharmaceutical dosage form using the following equation:

\[
C_{X} = C_{S} \left( \frac{V_{S}}{V_{X} + V_{S}} \right) \left(10^{(\Delta E / S)} - \frac{V_{X}}{V_{X} + V_{S}} \right)^{-1}
\]

Where \( C_{X} \) is the concentration to be determined, \( V_{S} \) is the volume of the original sample solution, \( V_{X} \) and \( C_{S} \) are the volume and concentration of the standard solution added to the sample to be analyzed, respectively, \( \Delta E \) is the change in potential after addition of certain volume of standard solution, and \( S \) is the slope of the calibration graph. Unknown drug concentration was determined from the graph produced by plotting the logarithm of CLO\(^{-}\) activity versus the potential.

In the potentiometric titrations different weights ranged from 2.09-41.99 mg of drug 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.

Determination of CLO in plavix

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

Content uniformity assay of Plavix tablets<905>

One tablet of Plavix (75 mg/tablet) was immersed in the measuring flask and adjusted to pH 1, 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 [36].

Determination of CLO in biological fluids

A. In serum: One ml of standard drug solution from 1×10⁻³, 1×10⁻⁴ and 1×10⁻⁵ M were added into a three of centrifugation 20-ml stoppered shaking tubes [34]. Each tube containing 9 ml of serum and 0.1 N acetate buffers was added to serum solution dropwise 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 deproteinated layer was transferred to a 100-ml measuring flask and complete to volume using bidistilled 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-H₂SO₄ was determined from the corresponding sensor calibration and standard addition methods.

B. 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 5 min, completed to the mark with doubly bidistilled 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 1.0×10⁻⁶ to 5.0×10⁻⁴ M drug. These solutions were subjected to the standard addition method for drug determination.

Dissolution <711>

One tablet of Plavix (75 mg/tablet) was placed in the vessel of instrument apparatus II. 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 0.816 in 1×10⁻³ solution.

| Ion-associate | Color   | Tentative Formulae | Found | Calc. | % | % | % |
|---------------|---------|--------------------|-------|-------|---|---|---|
| CLO₂-ST      | (off-white) | [C₁₆H₁₆ClN₀₂S]₃[SiW₁₂O₄₀] | 32.25 | 32.23 | 1.96 | 1.66 |
| CLO₂-SM      | (buff)   | [C₁₆H₁₆ClN₀₂S]₃[SiMo₁₂O₄₀] | 34.25 | 34.32 | 2.80 | 2.41 |
| CLO₂-PT      | (Y. white) | [C₁₆H₁₆ClN₀₂S]₃[PW₁₂O₄₀] | 17.40 | 17.45 | 1.44 | 1.25 |
| CLO₂-PM      | (faint yellow) | [C₁₆H₁₆ClN₀₂S]₃[PMo₁₂O₄₀] | 25.66 | 25.68 | 2.16 | 1.86 |
| CLO₂-TPB     | (White)  | [C₁₆H₁₆ClN₀₂S]₃[Cr(NH₃)₂(SCN)₄] | 64.93 | 64.94 | 4.80 | 4.87 |
| CLO-Rein     | (faint pink) | [C₁₆H₁₆ClN₀₂S]₃[Cr(NH₃)₂(SCN)₄] | 26.11 | 26.00 | 2.97 | 2.88 |

Table 1: Elemental analyses of the ion-associates.

Results 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 CLO₂-ST, CLO₂-SM, CLO₂-PT, CLO₂-PM, CLO₂-TPB and CLO₂-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, TBPs 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 5.0 wt% CLO₂-ST, 54.0 wt% graphite, and 41.0 wt% 2-NPPE exhibited the best response characteristics as 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].

Reproducibility of the sensor

The repeatability of the potential reading of the CLO₂-ST/CMCPS sensor was examined by subsequent measurements in 1.0×10⁻³ M CLO₂-H₂SO₄ solution immediately after measuring the first set of solution at 1.0×10⁻⁴ M CLO₂-H₂SO₄. The standard deviation for 5 replicate measurements of emf was found to be 0.473 in 1×10⁻⁷ M solution and 0.816 in 1×10⁻⁵ solution.

It was noticed that the slope of the calibration graph obtained by CLO₂-ST/CMCPS was nearly constant by polishing for any time taken 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 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 1.0×10⁻⁷ to 1.0×10⁻² M. In this study, practical response time was recorded by increasing CLO concentration by up to 10-fold. When the sensor was transferred from one concentration solution to another one, it was stabilized to a value higher than its value (8 s), which may be due to its memory effect. The sensor yielded steady potential within 10-12 s. 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.

### Table 2: Response characteristics of CLO-CMCPS at 95% confidence intervals at 25.0 ± 0.1°C.

| Interferent | MPMa | SSM | Interferent | MPMa | SSM |
|-------------|------|-----|-------------|------|-----|
| K⁺         | 2.88 | 3.15 | Cr³⁺        | 3.33 | 4.20 |
| Na⁺        | 2.95 | 3.33 | Maltose      | 3.52 | --- |
| NH₄⁺       | 2.65 | 2.93 | Glucose      | 3.50 | --- |
| Ba²⁺       | 3.21 | 3.55 | Lactose      | 3.54 | --- |
| Cu²⁺       | 3.20 | 3.62 | Urea         | 3.23 | --- |
| Co²⁺       | 3.11 | 3.55 | Ascorbic acid| 3.30 | --- |
| Ni²⁺       | 3.19 | 3.65 | Aspirin      | 1.15 | --- |
| Mn²⁺       | 2.56 | 3.11 | L-Lysine     | 3.34 | --- |
| Mg²⁺       | 2.89 | 3.36 | L-cystine    | 3.20 | --- |
| Zn²⁺       | 2.67 | 3.28 | L-Glycine    | 3.19 | --- |
| Fe³⁺       | 3.27 | 3.76 | L-Threonine  | 3.44 | --- |

*Interferent*: Each value is the average of three determinations.

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### Table 3: Composition and slope of calibration curves for different clopidogrel carbon paste electrodes at 25.0 ± 0.1°C.

| Composition % w/w | Ion-exchanger | graphite | 2-NPPE | Slope (mV/decade) | Linear range (M) |
|-------------------|---------------|----------|--------|------------------|-----------------|
| CLO4ST            | 1.0           | 54.0     | 45.0   | 55.5 ± 0.5       | 1.0 x 10⁻⁵-5.0x10⁻³ |
|                   | 3.0           | 54.0     | 43.0   | 58.9 ± 1.0       | 5.0 x 10⁻⁵-1.0x10⁻³ |
|                   | 5.0           | 54.0     | 41.0   | 60.0 ± 0.5*      | 1.0 x 10⁻⁵-1.0x10⁻³ |
| CLO4SM            | 1.0           | 54.0     | 45.0   | 48.7 ± 1.5       | 5.0 x 10⁻⁵-5.0x10⁻³ |
|                   | 3.0           | 54.0     | 43.0   | 57.9 ± 0.5       | 5.0 x 10⁻⁵-5.0x10⁻³ |
|                   | 5.0           | 54.0     | 41.0   | 56.3 ± 1.5       | 8.0 x 10⁻⁵-5.0x10⁻³ |
| CLO3PT            | 1.0           | 54.0     | 45.0   | 57.6 ± 1.0       | 5.0 x 10⁻⁵-1.0x10⁻³ |
|                   | 3.0           | 54.0     | 43.0   | 51.5 ± 1.0       | 5.0 x 10⁻⁵-5.0x10⁻³ |
|                   | 5.0           | 54.0     | 41.0   | 58.5 ± 0.5       | 1.0 x 10⁻⁴-1.0x10⁻³ |

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

**Figure 3:** Potential pH profile for different clopidogrel carbon paste electrodes at 25.0 ± 0.1°C.

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**Figure 4:** Effect of different plasticizers on the response of 5% CLO-ST.
mentioned pH ranges, the potential readings increase 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 sensor:** To study the thermal stability of the sensors, calibration graphs (emf, mV vs. pDrug) 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 \((dE_0/dt)_{cell}\), the standard cell potentials, \(E_0\), were determined at different temperatures from the respective calibration plots as the intercept of these plots at pDrug=0. Knowing that \(E_0\) is related to \((dE/dt)\) by the equation:

\[
E_0^\circ = E_0^{25\ ^\circ C} + (dE_0/dt)(t-25)^\circ C
\]

Plot of \(E_{e,0}\) 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 Figure 5b. 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 \(-0.0001\ \text{V}^\circ C^{-1}\) and \((dE_{cell}/dt)\) equals \(-0.0005\ \text{V}^\circ C^{-1}\). 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 clopidogrel cation. In the case of sugars and amino acids the high selectivity is related to the difference in polarity and lipophilic nature of their molecules relative to clopidogrel cation.

**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 & 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 and 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.

| Method | X ± S.E. | Relative error (%) | F<sup>1,3</sup> value (9.28) |
|--------|----------|--------------------|-----------------------------|
| Official method [32] | 98.0 ± 0.50 | 0.651 | 97.0 ± 0.6 |
| 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 |

Table 6: Statistical treatment of data obtained for the determination of CLO using the CLO-ST/CMCPE.
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 0.35 nM. 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 coformulated adjuvant as indicated by the mean recovery value of 99.87 ± 0.177 mV/decad for the investigated sensor.

Precision: The precision of the CMCPS method measured as percentage relative standard deviation (% RSD) 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%, 0.466% 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/urine using its prepared chemically modified carbon paste sensor.

Determination of Plavix tablet (75 mg/tablet): In order to assess the validity of the proposed sensor, the standard additions method [40], calibration curve method and the potentiometric titration method (Table 5 and Figure 6) 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 s), 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 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 1.0×10⁻² 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 6. 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 7 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/decade 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, 3503) was indicated by the results obtained as percentage was 99.83 ± 0.26 mV/decade 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-H2SO4 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 are a precision comparable to that of the pharmacopoeia 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 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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| Parameter | Ref [33] | Ref [34] [C.S] |
|-----------|----------|----------------|
|           | (PMA)    | (ARS)          | (PTA) CMCPS |
| o-NPOE    | 61.7     | 59.3           | 55.97       |
| DOP       | 55.97    | 0.9999         | 0.9999      |
| Correlation coefficient | 0.9874 | 0.9993         | 0.9999      |
| Linear range (M) | -- | -- | 1 x 10^-1 x 10^2 |
| LOD (M)   | 1.5 x 10^-6 | 5.01 x 10^-6 | 4.1 x 10^-6 |
| Working pH range | 1.5 - 4.0 | 1.2 - 4.6 | 1.2 - 4.8 |
| Response time (s) | 20 | 25 | 15 |
| Life span/days | 77 | 84 | 25 |
| Accuracy (%) | -- | -- | 99.09 ± 0.6 |
| Standard deviation | -- | -- | 99.72 ± 0.24 |
| Robustness | 99.48 ± 0.537 | 99.36 ± 0.337 | 99.51 ± 0.445 |
| Ruggedness | 99.51 ± 0.430 | 99.26 ± 0.650 | 98.77 ± 0.199 |

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