New electrodes for determination of Clopidogrel- bisulphate

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Abstract

In this research, new coated carbon electrodes were constructed and used for the determination of Clopidogrel drug. The electrodes were constructed by preparing ion-pair for (ClO⁻) with Silicotungstic acid (STA) using di butyl phthalate (DBP) and tri Butylphosphate as a Binder. These electrodes showed good sensitivity towards ClO⁻ with linear range of (1.0×10⁻⁷-1.0×10⁻³) M for both, correlation coefficient, (0.9693 and 0.9881) for DBP and TBP respectively, the life time (25 and 23) days, optimum temperature range (25-55)°C and optimum pH range (1.4-3.7) for both electrodes, limit of detection (3.33×10⁻⁸ and 2.63×10⁻⁸) M, slope (30.19 and 24.08) mV/decade for CLO-AST DBP and TBP electrodes respectively. These electrodes were successfully applied for determination of CLO in pure and pharmaceutical preparation form (tablets) with recovery of 99.42 , 99.03 % for DBP and TBP respectively and Biological sample (Uria recovery of 99.47 , 99.24 % and serim with recovery of 99.26 , 98.72 % for both DBP, TBP.

Key words: Coated wire electrode, Clopidogrel drug, Potentiometry, nanosilicate

Introduction

Clopidogrel bisulfate 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].
Clopidogrel hydrogen sulfate, methyl (+)-(S)-(ochlorophenyl)-6,7-dihydrothieno[3,2-c]pyridin-5(4H)-acetate hydrogen sulfate, is a novel thienopyridine derivative that irreversibly blocks adenosine diphosphate (ADP) and is important in platelet aggregation, the cross-linking of platelets by fibrin. Clopidogrel (Fig. 1) is chemically related to Ticlopidine with superior side effects profile and dosing requirements [5-7].

![Fig. (1). Structural formula of clopidogrel](image)

The empirical formula of clopidogrel bisulfate is \( C_{16}H_{16}ClNO_2S\cdot H_2SO_4 \) and its molecular weight is 419.9 [8]. The molecule of clopidogrel contains an asymmetrical carbon leading to the existence of two enantiomers (R and S) [9, 10]. Clopidogrel free base was unstable due to a labile proton in the chiral center and was susceptible to racemization and hydrolysis of methyl ester group [11, 12].

Different analytical methods for the determination of clopidogrel were used such as spectrophotometric [11-16] techniques, gas chromatography–mass spectrometry (GC–MS) [17], liquid chromatography–mass spectrometry (LC–MS) [18,19], HPLC–ESI-MS/MS, [20] RHPLC with UV detection [21-25], HPTLC [26,27]. Furthermore, the determination of clopidogrel and its impurities by capillary electrophoresis was reported [28-30] and also by voltammetry [31].

This work describes the fabrication, construction with sensor potentiometric characterization, and application of a novel clopidogrel chemically modified PVC Silicon Oxide nanoparticles (CLO-NSiOPs) using clopidogrel-silicotungstate (CLO-STA).

**Reagents and materials**

All chemicals were of analytical reagent grade. Distilled water was used for the preparation of stock solutions. The following materials: CLO (India-) Poly vinyl chloride (PVC) high molecular weight (Fluka), Di butyl phthalate (Sigma, Germany), Tetra hydro furan.(THF) (Aldrich, USA). Silicotungstic acid (Merck, Germany) were used in this work. A stock solution of 0.01 M of CLO drug was prepared by
dissolving 4.199 g in 100 ml distilled water. Dilute solutions (1x10^{-9} to 1x10^{-3} ) M of drug were prepared by appropriate dilution with distilled water.

**Apparatus**

Potentiometric measurements were performed using HANNA instruments 301 pH meter, Jenway 3345 pH meter, calomel electrodes (RE-2BH). For elemental analysis CHN Elementar Isoprime 100-Germany was used.

**Sample preparation**

Fourteen tablets of the Plavineer (75 mg/ tablet) were finely powdered, the average weight of tablets is (3.832 g) and the weight of (1.5324 g) of this tablet powder (equivalent to 0.4199g CLO) was dissolved in 100 ml distilled water. And ten tablets of Plavineer (75 mg/ tablet) were finely powdered (the average weight of tablets is 2.5656 g). An accurate weight amount 1.4363 g of this powder (equivalent to 0.4199 g CLO) was dissolved in 100 ml distilled water. Seven tablets of Clopidogrel Film-coated (75 mg/ tablet) were finely powdered (the average weight of tablets 1.9583 g) an accurate weight of amount 1.5662 g of this powder (equivalent to 0.4199 g CLO) was dissolved in 100 ml distilled water. These three tablet solutions were filtered and to obtain 1.0x10^{-2} mol/l CLO solution. The working solutions in the range of 1.0 x10^{-7} - 1.5 x 10^{-2} mol/l were prepared by dilutions using distilled water.

**Stock Solutions of Interfering Ions:**

Solutions of 1.0x10^{-3} mol L^{-1} for each of NaCl, KCl, BaCl_2, MgCl_2, MnCl_2, CrCl_3, FeCl_3, NH_4Cl, CuCl_2, ZnCl_2, NiCl_2, CoCl_2, Tartarate, Glucose, D(-)-Fructose, Lactose, Uric acid, Ascorbic acid, Aspirin were prepared by dissolving appropriate amount of these materials in distilled water in 50ml volumetric flasks.

**Preparation of ion-pairs**

The ion-pair was prepared by mixing 50 mL of equimolar of 1.0 x 10^{-2} M CLO to 50 mL of STA a white precipitate of CLO-STA was formed. The precipitate was filtered through Whatman filter paper
(No. 42), and washed several times with distilled water. The precipitate was left for 24 h to dry at room temperature.

**Preparation of modified coated wire sensor:**

Copper wire of 10 cm length and 1.0 mm diameter insulated by tight polyethylene tubes leaving 0.5 cm at one end for connection and at the other end 1 cm for coating. The coating mixture was prepared by dissolving 0.01 g ion pair (CLO-STA) in the minimum possible amount of tetrahydrofuran (2-3 ml) with 0.19 g powdered PVC and 0.35 ml of TBP or DBP as plasticizer and 0.01 g of Nanosilica particles was added. Before coating the polished surface of the copper wire was washed with HNO$_3$ followed by distilled water, and dried with acetone. The polished surface of the wire was coated with the active membrane by dipping (1 cm for coating) of this end into the coating solution then the film left for about 3 minutes for dryness. The process was repeated for eight times. The prepared CWE were soaked in 1x10$^{-3}$ M solution of the CLO for 24 h.

**Results and Discussion:**

CLO-STA as an electro active compound was used to prepare new sensors. Elemental analysis was carried out to confirm the composition of the ion-pair (CLO-STA). The obtained result revealed 9:1 composition for [CLO:STA] ion pair as indicated in (table 1)

(Table 1): Elemental analysis of the (CLO-STA) ion pair

| Sample       | Color | Formula                  | %C       | %H       | % N       |
|--------------|-------|--------------------------|----------|----------|----------|
| (CLO)$_9$STA | white | [C$_{16}$H$_{16}$O$_3$SN]$_9$ | found    | 28.19    | 2.45     | 2.059    |
|              |       | [H$_4$SiW$_{12}$O$_{40}$].2H$_2$O | Calculate | 28.134   | 2.47     | 2.051    |
The IR Spectrum of pure CLO, the peaks at (2954, 2877, 2552, 1752, 1188) cm\(^{-1}\) are assigned for (-CH\(_2\), -CH\(_3\), C-S-C, C=O ester, C-O ester) respectively.

In the Spectrum of (CLO)\(_9\)STA ion pair, the peaks at (1745, 1619, 1080) cm\(^{-1}\) are assigned for (C=O carboxylic, C=C, C-O ester) respectively. The disappearance of (C=O, C-O ester and C-S-C) is attributed to the occurrence of hydrogen bonding which is assured by the broad band at 3399 cm\(^{-1}\) in figure (2).

**The Effect of Binder**

The influence of the binder type on the characteristics of the studied sensors was investigated by using two binders of different polarities (DBP and TBP) and the results of this study are shown in (table 2).

**Table (2)** Slope and R\(^2\) values for the calibration curves for NSiOS with different binders at 25.0 °C.

|       | DBP     | TBP     |
|-------|---------|---------|
| Slope | 30.19   | 24.08   |
| R\(^2\) | 0.9693  | 0.9884  |

(NSiOS) range (10\(^{-7}\) - 10\(^{-3}\)) M
The sensor with DBP as a solvent mediator gives the best response for pure drug and the best sensors are found to be in DBP binder because the slope of calibration is better.

**Reproducibility of the Sensors**

The results of the two modified sensors NSiOS is compared with coated wire sensor CWS. The repeatability of the sensor response was examined by measurements in the range $1.0 \times 10^{-7}$ - $1.0 \times 10^{-3}$ M ClO solution in DBP and TBP binder and the SD for 5 replicate for NSiOS electrode measurements of emf was found to be (1.088, 0.95) for DBP and TBP binder respectively.

**Response Time and Life Time**

The dynamic response time of sensor was tested by measuring the time required to achieve a steady-state potential (within $\pm 1$ mV) after successive immersions of the sensor in a series of drug solutions, each having a 10-fold increase in concentration from $1.0 \times 10^{-7}$ - $1.0 \times 10^{-3}$ M. When the sensor was transferred from one concentration solution to another one, it was stabilized to a value higher (17s) than its value, which may be due to the memory effect. Steady potential within of (17 and 25) s for NSIO sensors for DBP and TBP respectively show in (table 2).

The life time is (25, 23) day for NSiOS sensors in (DBP and TBP) binder (table 3).

**Table (3)** Electrochemical response Characteristics of NSiOS in DBP and TBP.

| Parameter               | DBP | TBP |
|-------------------------|-----|-----|
|                         | NSiOS | NSiOS |
| Slope (mV decade$^{-1}$) | 30.19 | 24.08 |
| Intercept               | 256.231 | 218.6 |
| Correlation Coefficient $R^2$ | 0.9693 | 0.9881 |
Calibration plot of the fabricated electrodes and limit of detection

The fabricated electrodes (NSiOS-DBP) and (NSiOS-TBP) were immersed along with Calomel reference electrode in solutions of CLO in the concentration range (1x10^{-8} - 1x10^{-2}) M. The E (mV) against –log [CLO] was plotted as shown in figure 3. Both electrodes show a linear response over the concentration ranges from (1x10^{7} - 1x10^{-3}) M with near Nernstian slopes of and (30.19 and 24.08) mV /decade for (NSiOS-DBP) and (NSiOS-TBP), and values of LOD (3.33x10^{-8} and 2.63x10^{-8}) for (NSiOS-DBP) and (NSiOS-TBP) electrodes respectively indicating that the sensors under investigation are highly sensitive which can be applied for determination of trace amounts of CLO drug it shown in (table 3).

|                           | Linear range (mol./L) | limit of detection (M) | Response time for S | Working pH range | Life time, day | Temperature °C | Accuracy (% recovery) |
|---------------------------|-----------------------|------------------------|---------------------|------------------|----------------|-----------------|----------------------|
|                           | 10^{-7}–10^{-3}       | 3.33x10^{-8}           | 25                  | 1.4 – 3.7        | 25             | 25-55           | 99.42 ± 1.088        |
|                           | 10^{-7}–10^{-3}       | 2.63x10^{-8}           | 17                  | 1.4 - 3.7        | 23             | 25-55           | 99.03 ± 0.95         |

Figure (3): Standard curve of CLO using new (NSiOS-DBP) and (NSiOS-TBP) electrodes.
Effect of pH

The effect of pH on the performance of the two electrodes was investigated using concentration of (1.0×10^{-4})M of CLO drug at different pH values (1-10.5). The pH value was adjusted by addition of small volumes of HCl and/or NaOH solution (0.1-1 M of each). The potential at each pH value was recorded. It is obvious (fig 4) that the best pH range is from (1.4-3.7) for both electrodes where the potential is independent on pH. However the potential decreases gradually at pH values higher than 3.7 for (NSiOS-DBP) and (NSiOS-TBP) it shown in (table 3) and (figure 4).

![Figure 4: Effect of pH on the response of NSiOS-DBP and NSiOS-TBP electrodes using CLO solution (1×10^{-4})M](image)

Selectivity of the studied sensors

The selectivity coefficients (log K) of the studied sensors were determined applying separate solution method (SSM) [32, 33]. In SSM, the potential of cell comprising the new constructed electrode and a reference electrode is measured in two separate solutions, where (CLO) and (interfering ion or compound) are at the same activity. Selectivity coefficients were calculated using Nicolsky equation [32] :

$$\log K = (E_j - E_i)/S$$
where $E_i$ is the potential measured in $(1 \times 10^{-3})$ mol/L CLO, $E_j$ the potential measured in $1 \times 10^{-3}$ mol/L of the interfering ion or compound, $S$ is the slope of the calibration plot. The $K$ value represents the difference in potential in the presence of interfering ion ($j$) and when ($j$) is not present. When the value is less than 1 this indicates that the electrode shows low response to the interfering ions. The results of selectivity are shown on table (4). The $K$ values show a very high selectivity of the electrodes towards the CLO.

Table (4): $K$ value of both NSiOS-DBP and NSiOS-TBP electrodes

| Interfering ion or compound | NSiOS-DBP         | NSiOS-TBP         |
|-----------------------------|-------------------|-------------------|
| $K^+$                       | $4.51 \times 10^{-3}$ | $4.42 \times 10^{-3}$ |
| Na$^+$                      | $3.82 \times 10^{-3}$ | $3.52 \times 10^{-3}$ |
| NH$_4^+$                    | $3.95 \times 10^{-3}$ | $3.75 \times 10^{-3}$ |
| Ba$^{2+}$                   | $3.34 \times 10^{-3}$ | $3.12 \times 10^{-3}$ |
| Cu$^{2+}$                   | $4.13 \times 10^{-3}$ | $4.13 \times 10^{-3}$ |
| Co$^{2+}$                   | $2.10 \times 10^{-3}$ | $2.40 \times 10^{-3}$ |
| Ni$^{2+}$                   | $3.34 \times 10^{-3}$ | $3.55 \times 10^{-3}$ |
| Mn$^{2+}$                   | $2.12 \times 10^{-3}$ | $2.02 \times 10^{-3}$ |
| Mg$^{2+}$                   | $3.32 \times 10^{-3}$ | $3.01 \times 10^{-3}$ |
| Zn$^{2+}$                   | $1.95 \times 10^{-3}$ | $1.63 \times 10^{-3}$ |
| Fe$^{3+}$                   | $2.14 \times 10^{-2}$ | $2.01 \times 10^{-2}$ |
| Cr$^{3+}$                   | $4.30 \times 10^{-3}$ | $4.12 \times 10^{-3}$ |
| Tartrate                    | $3.18 \times 10^{-2}$ | $3.23 \times 10^{-2}$ |
| D- Fructose                 | $2.51 \times 10^{-3}$ | $2.31 \times 10^{-3}$ |
### Glucose

| Glucose     | 4.52 x 10^{-3} | 4.42 x 10^{-3} |
|-------------|----------------|----------------|

### Lactose

| Lactose     | 3.43 x 10^{-2} | 3.61 x 10^{-2} |
|-------------|----------------|----------------|

### Uric acid

| Uric acid   | 3.34 x 10^{-2} | 3.66 x 10^{-2} |
|-------------|----------------|----------------|

### Ascorbic acid

| Ascorbic acid | 3.33 x 10^{-2} | 3.43 x 10^{-2} |
|---------------|----------------|----------------|

### Aspirin

| Aspirin      | 2.21 x 10^{-2} | 2.00 x 10^{-2} |
|--------------|----------------|----------------|

**Precision and Accuracy**

To evaluate the accuracy and precision of the proposed method, pure and pharmaceutical drug solutions at different levels (within the working limits) was analyzed, each solution was repeated for (5) times. Precision and accuracy were based on the calculated percent relative standard deviation (RSD%) and percent relative error (RE %). The value of RE% is not more than (-0.97) and value or RSD% is not more than (1.56). The results (table 6) show that these methods have reasonable precision and accuracy.

**Robustness and Ruggedness**

The robustness method of the NSiOS was examined by changing the aqueous solution to acetate buffer pH (4) and the percentages recovery were 99.2 ± 1.8 for the NSiOS in DBP binder and 98.6 ± 1.45 in the TBP binder.

The ruggedness or the reproducibility was checked by using another model of pH-meter (Hanna) and different calomel (Jenway) electrode. The results obtained as percentage recovery was 99.0 ± 1.3 for the NSiOS in DBP binder and 99.2 ± 1.6 in TBP binder for Hanna instrument but when we using Jenway reference electrode the recovery are 99.4 ± 1.9 for the NSiOS in DBP binder and 98.2 ± 1.3 for the NSiOS in TBP binder (table 5).

These results were closely in agreement with those obtained for standard drug solution, using new conditions for robustness and ruggedness tests (Table 6, Table 5).
This is main two sensor give accurate result when we change the instrument and medium.

(Table 5) The recovery ± SD For robustness and ruggedness for NSiOs in TBP and DBP binder

| Parameter                                      | NSiOS - TBP   | NSiOS - DBP   |
|------------------------------------------------|---------------|---------------|
| Robustness                                     | 98.6±1.45     | 99.2±1.8      |
| Ruggedness for (Hanna pH meter)                 | 99.2±1.6      | 99.0±1.3      |
| Ruggedness for (Jenway calomel reference electrode) | 98.2±1.3      | 99.4±1.9      |

Analytical Applications:

CLO was determined using a direct calibration method for both pure and pharmaceutical forms. The results were calculated as the recoveries % using the prepared electrodes (NSiOS-DBP) and (NSiOS-TBP). The percentage recoveries for determination of CLO pure drug solutions are (99.19 and 99.03) for (NSiOS-DBP and NSiOS-TBP) electrodes respectively. The percentage recoveries for determination of CLO tablets (Plavinerr 75 mg tablet) are (99.42 and 99.48) and for (Plagerine 75 mg tablet) are (99.89 and 99.1) but for (Clopidogrel 75 mg tablet) are (99.98 and 99.44) for (NSiOS-DBP and NSiOS-TBP) electrodes respectively shown in (table 6).
Table (6) Statistical treatment of data for determinations of CLO in pure and pharmaceutical preparations using new (NSiOS-DBP) and (NSiOS-TBP) electrodes

| Sample                  | NSiOS-DBP | TBP | NSiOS-TBP | TBP |
|-------------------------|-----------|-----|-----------|-----|
|                         | Taken log [CLO] M | Found | % recovery | Taken log [CLO] M | Found | % recovery |
| Pure drug               | 7.0       | 6.90 | 98.57     | 7.0  | 6.99 | 99.86     |
|                         | 6.0       | 5.97 | 99.5      | 6.0  | 5.95 | 99.17     |
|                         | 5.0       | 4.95 | 99.00     | 5.0  | 4.85 | 97.00     |
|                         | 4.0       | 3.91 | 97.75     | 4.0  | 4.01 | 100.25    |
|                         | 3.0       | 3.01 | 100.33    | 3.0  | 2.99 | 99.67     |
|                         | 99.03 ±0.95 |      |           | 99.19 ± 1.46 |      |           |
| % Mean± SD.             | n 5       |      |           | n 5   |      |           |
|                         | Variance  | 0.90 |           | 2.13  |      |           |
|                         | % RE      | 0.96 |           | - 0.81 |      |           |
|                         | % RSD     | 1.47 |           | 1.47  |      |           |
| Plavineer Iraq 7.0      | 7.0       | 7.12 | 101.71    | 7.0  | 7.05 | 100.71    |
| Hawlear 75 mg, 14 tablet| 6.0       | 5.92 | 98.67     | 6.0  | 5.99 | 99.83     |
|                         | 5.0       | 4.91 | 98.2      | 5.0  | 4.89 | 97.8      |
|                         | 4.0       | 4.02 | 100.5     | 4.0  | 3.99 | 99.75     |
|                         | 3.0       | 2.95 | 98.33     | 3.0  | 2.97 | 99.00     |
|                         | 99.48 ±1.55 |      |           | 99.42 ±1.088 |      |           |
| % Mean± SD.             | n 5       |      |           | n 5   |      |           |
|                         | Variance  | 2.4  |           | 1.18  |      |           |
|                         | % RE      | - 0.52 |      | - 0.58 |      |           |
|                         | % RSD     | 1.56 |           | 1.094 |      |           |
| Plagerine (75 mg, 10 tablet) | 7.0  | 7.07 | 101.0     | 7.0  | 7.1  | 101.43     |
| Indi                    | 6.0       | 5.89 | 98.17     | 6.0  | 5.99 | 99.83     |
|                         | 5.0       | 4.90 | 98.00     | 5.0  | 4.95 | 99.00     |
|                         | 4.0       | 3.96 | 99.0      | 4.0  | 3.98 | 99.5      |
|                         | 3.0       | 2.98 | 99.33     | 3.0  | 2.99 | 99.67     |
|                         | 99.1 ±1.2 |      |           | 99.89 ± 0.92 |      |           |
| % Mean± SD.             | n 5       |      |           | n 5   |      |           |
|                         | Variance  | 1.44 |           | 0.85  |      |           |
|                         | % RE      | - 0.9 |      | - 0.11 |      |           |
|                         | % RSD     | 1.21 |           | 0.921 |      |           |
| Clopidogrel film-coated | 7.0       | 7.05 | 100.7     | 7.0  | 7.02 | 100.28     |
|                         | 6.0       | 6.01 | 100.16    | 6.0  | 6.05 | 100.83     |
Analytical Applications

The standard addition, calibration curve and potentiometric titration methods were proved to be successful for the determination of CLO in its pure solutions, CLO tablets (75 mg/tablet, table 6) and biological fluids (human serum and urine, table 7) using the NSiOS.

Determination of CLO in Human Serum and Urine:

The proposed GGOS, CWS and NSiOS methods were successfully applied to determine CLO in biological fluids and the obtained results are summarized in Table (7).

The determination of CLO in spiked human serum shows that a wide concentration range of the drug can be determined by the proposed sensors with high precision and accuracy.

In urine samples the standard addition method was applied to overcome the matrix effects in these samples. The sensors are washed by distilled water after each measurement to protect the sensor surface from sticking of biological components. In spiked urine and human serum the recovery was ranged from 99.47-99.26 (table 7) using DBP binder and 99.47-99.26 for NSiOS show in (Table 7) using TBP binder.

Table (7) Statistical treatment of data obtained for determination CLO in Human urine and serum using NSiOS) – for both DBP and TBP binder

| Sample        | NSiOS-TBP                        | NSiOS-DBP                        |
|---------------|----------------------------------|----------------------------------|
|               | Taken -log [CLO] M | Found | % recovery | Taken -log [CLO] M | Found | % recovery |
| Human Urine   | 7.0 | 7.03 | 100.43 | 7.0 | 7.02 | 100.29 |
|               | 6.0 | 5.9  | 99.12  | 6.0 | 5.97 | 99.50  |
|               | 5.0 | 4.95 | 99.00  | 5.0 | 4.99 | 99.80  |
|               | 4.0 | 3.92 | 98.00  | 4.0 | 3.95 | 98.75  |
|               | 3.0 | 2.99 | 99.67  | 3.0 | 2.97 | 99.00  |
Conclusion

The proposed method introduced an ion selective electrodes for the determination of CLO based on PVC matrix and (DBP and TBP) as a plasticizer and using STA as active materials. These electrodes showed a successful application with low limit of detection and good recovery. The electrodes also showed long life time, fast response time, good selectivity and reasonable working concentration ranges.

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