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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 Binderr. 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)^{o}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 respectivllyand 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 hydrogensulfate, 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].

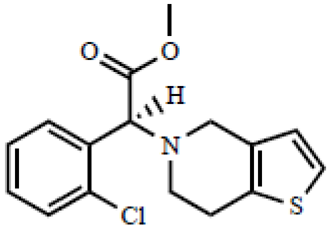


Fig. (1). Structural formula of clopidogrel

The empirical formula of clopidogrel bisulfate is $C_{16}H_{16}ClNO_2S{\bullet}H_2SO_4$ 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 instruments301 pH meter, Jenway3345 pH meter , calomel electrodes (RE-2BH). For elemental analysis CHN ElementarIsoprime100-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 to0.4199g CLO) was dissolved in 100 ml distilled water. And ten tablets of Plavineer (75 mg/ tablet) were finely powdered(the average weights oftablets is2.5656 g).An accurate weight amount 1.4363 gof this powder(equivalent to0.4199 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 tablets1.9583 g) an accurate weight of amount 1.5662 g of this powder(equivalent to0.4199g CLO)was dissolved in 100 ml distilled water. These three tablet solutions were filtered and to obtain 1.0×10^{-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 ofInterfering 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:

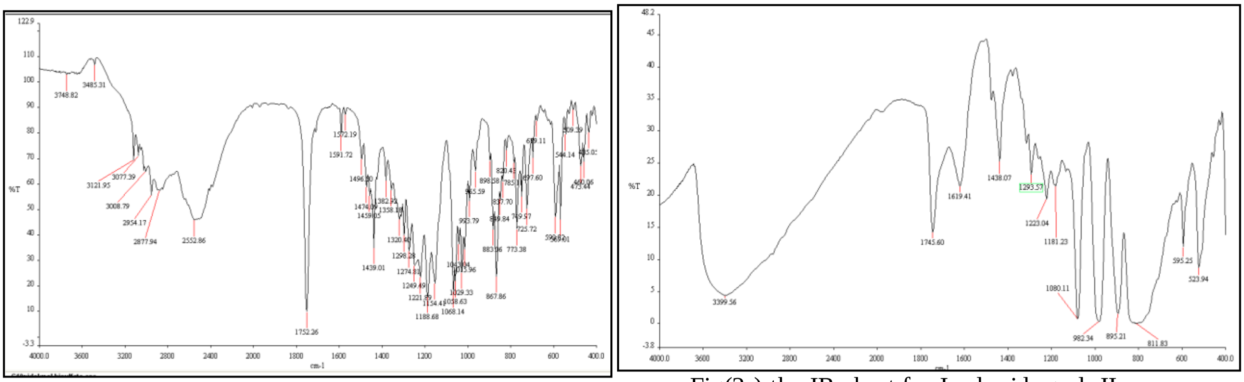
Copper wire of 10 cm length and 1.0 mm diameter insulated by tight polyethylene tubes leaving 0.5 cm at one end for connection and at the other end 1 cm for coating. The coating mixture was prepared by dissolving 0.01 g ion pair (CLO-STA) in the minimum possible amount of tetra hydrofuran (2-3 ml) with 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 withHNO_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 minutesfor dryness. The process was repeated for eighttimes. The prepared CWE were soaked in $1x10^{-3}$ M solution of the CLO for 24h .

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
$(CLO)_9STA$	white	$[C_{16}H_{16}O_3SN]_9$	found	28.19	2.45	2.059
		$[H_4SiW_{12}O_{40}].2H_2O$	Calculate	28.134	2.47	2.051



Fig(2) the IR chart for I- clopidogrel ,II- clopidogrel - tungstosilicic acid haydrate

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 $(CLO)_9STA$ ion pair , the pecks at (1745,1619, 1080) 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 slop of calibration is beter.

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 range1.0 x 10^{-7} - 1.0 x 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 ± 1 mV) after successive immersions of the sensor in a series of drug solutions, each having a 10-fold increase in concentration from 1.0x10^{-7} - 1.0x10^{-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 respectivly show in (table 2).

The life time is (25,23) day for NSiOS sensors in (DBP and TBP) binder (table3).

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

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

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 figure3. 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).

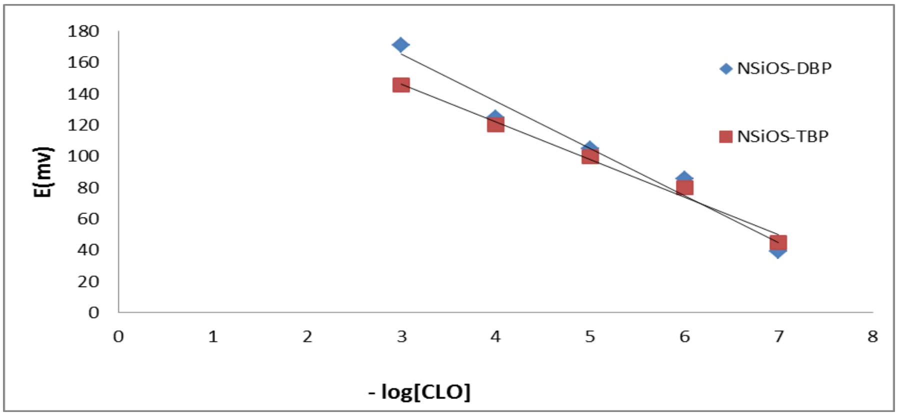


Figure (3): Standard curve of CLO using new (NSiOS-DBP) and (NSiOS-TBP) electrodes.

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).

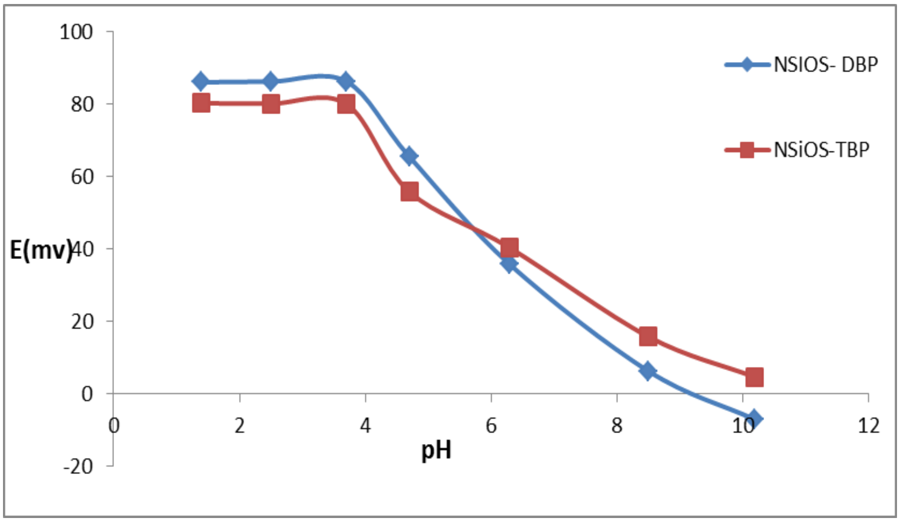


Figure (4): Effect of pH on the response of NSiOS-DBP and NSiOS-TBP electrodes using CLO solution (1×10^{-4})M

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 Ei is the potential measured in ($1x10^{-3}$) mol/L CLO ,Ej the potential measured in $1x10^{-3}$ 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 shows 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	K	
	NSiOS-DBP	NSiOS-TBP
K^+	4.51×10^{-3}	4.42×10^{-3}
Na^+	3.82×10^{-3}	3.52×10^{-3}
NH_4^+	3.95×10^{-3}	3.75×10^{-3}
Ba^{2+}	3.34×10^{-3}	3.12×10^{-3}
Cu^{2+}	4.13×10^{-3}	4.13×10^{-3}
Co^{2+}	2.10×10^{-3}	2.40×10^{-3}
Ni^{2+}	3.34×10^{-3}	3.55×10^{-3}
Mn^{2+}	2.12×10^{-3}	2.02×10^{-3}
Mg^{2+}	3.32×10^{-3}	3.01×10^{-3}
Zn^{2+}	1.95×10^{-3}	1.63×10^{-3}
Fe^{3+}	2.14×10^{-2}	2.01×10^{-2}
Cr^{3+}	4.30×10^{-3}	4.12×10^{-3}
Tartrate	3.18×10^{-2}	3.23×10^{-2}
D- Fructose	2.51×10^{-3}	2.31×10^{-3}

Glucose	4.52×10^{-3}	4.42×10^{-3}
Lactose	3.43×10^{-2}	3.61×10^{-2}
Uric acid	3.34×10^{-2}	3.66×10^{-2}
Ascorbic acid	3.33×10^{-2}	3.43×10^{-2}
Aspirin	2.21×10^{-2}	2.00×10^{-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. was presented by the results obtained as percentage recovery was 99.0 ± 1.3 for the NSiOS in DBP binder and .9, 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 andnpharmaceutical preparations using new (NSiOS-DBP) and (NSiOS-TBP) electrodes

Sample	NSiOS- TBP			NSiOS- DBP		
	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
% Mean± SD.	99.03 ±0.95			99.19 ± 1.46		
n	5			5		
Variance	0.90			2.13		
% RE	- 0.97			- 0.81		
% RSD	0.96			1.47		
Plavineer Iraq Hawlear 75 mg , 14 tablet	7.0	7.12	101.71	7.0	7.05	100.71
	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
% Mean± SD.	99.48 ±1.55			99.42 ±1.088		
n	5			5		
Variance	2.4			1.18		
% RE	- 0.52			- 0.58		
% RSD	1.56			1.094		
Plagerine (75 mg, 10 tablet) Indi	7.0	7.07	101.0	7.0	7.1	101.43
	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
% Mean± SD.	99.1 ±1.2			99.89 ± 0.92		
n	5			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

Tablet (75mg, 7 tablet), UK	5.0 4.0 3.0	4.93 3.95 2.97	98.60 98.75 99.00	5.0 4.0 3.0	4.99 3.98 2.985	99.80 99.50 99.50
% Mean± SD.	99.44± 0.93			99.98 ± 0.57		
n	5			5		
Variance	0.86			0.32		
% RE	- 0.56			- 0.02		
% RSD	0.93			0.57		

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 6.0 5.0 4.0 3.0	7.03 5.9 4.95 3.92 2.99	100.43 99.12 99.00 98.00 99.67	7.0 6.0 5.0 4.0 3.0	7.02 5.97 4.99 3.95 2.97	100.29 99.50 99.80 98.75 99.00

% Mean± SD.	99.24 ± 0.90			99.47 ± 1.51		
n	5			5		
Variance	0.81			2.28		
% RE	- 0.76			- 0.53		
% RSD	0.91			1.52		
Human Serum	7.0	6.89	98.43	7.0	7.04	100.57
	6.0	6.09	101.50	6.0	5.96	99.33
	5.0	4.87	97.4	5.0	4.92	98.4
	4.0	3.97	99.25	4.0	3.96	99.00
	3.0	2.91	97.00	3.0	2.97	99.00
% Mean± SD.	98.72 ± 1.79			99.26 ± 1.40		
n	5			5		
Variance	3.20			1.27		
% RE	- 1.28			- 0.74		
% RSD	1.81			1.41		

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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