Determination of Clopidogrel bisulphate Using Ion-Selective Electrodes in Bulk, Pharmaceutical Formulation and in Biological Fluids

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Abstract: The construction and performance characteristics of clopidogrel bisulphate (CLP) selective electrodes were developed. Three types of electrodes: plastic membrane I, coated wire membrane II and coated graphite III, electrodes were based on the incorporation of CLP with the pairing agents phosphomolybdic acid (PMA), ammonium reineckate salt (ARS), and phosphotungstic acid (PTA) respectively. The electrodes displayed a Nernstian response with a mean calibration graphs slopes of 55.97±0.460, 57.57±0.227 and 58.03±0.150 mV decade^{-1} for the three electrodes respectively, over linear concentration range 1.0 x 10^{-7} - 1.0 x 10^{-2} mol L^{-1} of the drug, with detection limits 5.01 x 10^{-8}, 4.10 x 10^{-8} and 5.00 x10^{-8} mol L^{-1} for electrodes I, II and III, respectively. The safe pH range of the proposed electrodes was (1.2- 4.6). The influence of possible interfering species such as inorganic cations, sugars and amino acids was studied. The results were favorably compared to those obtained by a reference method. The proposed electrodes were used for the determination of CLP in pure form, pharmaceutical formulation and in biological fluids.

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1. Introduction

Clopidogrel bisulphate (CLP), methyl(+)-(s)-α-(o-chlorophenyl)6,7-dihydrothieno (3, 2-c) pyridine-5(4H)- acetate bisulphate (Figure 1), is a new anti platelet agent, and it is similar to ticlopidine in chemical structure [1,2]. It prevents ischemic stroke, mycordil infraction and vascular disease [3]. It inhibits platelet aggregation by selective preventing binding adenosine diphosphate (ADP) to its platelet receptor [4].

For this drug the literature reveals a variety analytical methods such as UV spectrophotometric methods for estimation of CLP [5-9], kinetic spectrophotomety for determination of CLP, petaxolol and imidril in pharmaceutical formulation [10]. Other methods included: nonenzymatic and enenzymatic chiral inversion of CLP using ^{1}H-NMR and a chiral HPLC procedure [11]. GC-MS for the analysis of carboxylic acid metabolite of CLP in plasma and serum [12], reverse phase HPLC with UV detection for the determination of CLP in pharmaceutical preparations has been reported [13-17], HPTLC [18,19] and HPLC [20-22]. CLP was determined in presence of its metabolite in human plasma by LC coupled with mass spectrometry [23,24], and HPLC-MS/MS [25]. Also capillary electrophoresis methods were reported [26-28], and voltammetry [29].

Ion-selective electrodes have many advantages relation to other analytical techniques, being accurate, fast, economic, simple and sensitive also have an extremely wide range of applications in agriculture, industrial fields, medicinal and water pollution. The aim of this work was to develop a sensitive, selective and validated three types of selective electrodes for the determination of CLP in bulk, pharmaceutical formulations and in biological fluids.

2. Experimental

2.1. Materials and reagents

All chemicals used in this work were of analytical grade, Pure grade CLP was provided from Saudi Pharmaceutical Industries & Medical Application Corporation (SPIMACO), Al-Qassim pharmaceutical Plant. While pharmaceutical preparation (Plavix® 75mg/tablet) was provided by (Sanofi Aventis). Methanol 99.0%, acetone 99.9%, tetrahydrofuran (THF) 97.0% and dibutyl phethalate (DBP) were provided by Fluka. Phosphomolybdic acid (PMA), ammonium reineckate (ARS), phosphotungstic acid (PTA) and polyvinyl chloride (PVC) high molecular weight were purchased from Aldrich, Germany. Urine samples were obtained from healthy volunteers and serum samples were obtained from (united diagnostics industry).

2.2. Standard drug solution

A stock solution 1x10^{-1} mol L^{-1} was prepared by dissolving 1.0 g of CLP in 25 mL of distilled water pH 1. Serial dilutions were prepared using distilled water.

2.3. Preparation of ion-pairs

The ion-pair clopidogrel-phosphomolybdate (CLP-PMA) (faint yellow powder), clopidogrel-ammonium

reineckate (CLP-ARS) (faint pink powder) and clopidogrel-phosphotungustate (CLP-PTA) (faint yellowish white powder) were prepared by the addition of 50 mL of 1.0 x 10^{-2} mol L^{-1} CLP solution to 50 mL of 1.0× 10^{-2} mol L^{-1} of (PMA), (ARS) and (PTA). The precipitates were filtered, washed thoroughly with distilled water and dried at room temperature for 24 hrs.

2.4. Membrane composition

The membranes were prepared by dissolving required amount of ion-pair, 190 mg of powdered polyvinyl chloride (PVC) and plasticizer di-butylphthalate (DBP) in 5 mL tetrahydrofuran (THF). The solution was poured into a Petri dish (3 cm in diameter), covered with a filter paper and the solvent was allowed to evaporate slowly at room temperature.

2.5. Electrode construction

Plastic membrane electrode: A punched circular membrane was attached to a poly-ethylene tube in an electrode configuration by means of PVC-THF solution. A mixture containing equal volumes of 1.0×10^{-3} mol L^{-1} CLP and potassium chloride was used as internal reference solution in which the Ag/AgCl reference electrode was dipped. The constructed electrode was pre-conditioned after preparation by soaking for 24 hrs in $1.0x10^{-3}$ mol L^{-1} drug solution and stored in the same solution.

Coated wire electrode: Pure aluminum wire of 11.0 cm length was tightly insulated by polyethylene tube leaving 2.0 cm at one end for the coating and 1.0 cm at the other end for connection. Prior to coating, the polished aluminum surface was washed with a detergent, thoroughly rinsed with water, and dried. Afterwards, the aluminum wire was coated by quickly dipping it into the coating solution several times and allowing the film left on the wire to dry for about 3 min. The prepared electrode was conditioned by soaking for 24 hrs in 1.0 x 10^{-3} mol L^{-1} CLP solution. All potentiometric measurements were performed using the following cell assembly: Al / membrane / test solution // KCl salt bridge // SCE.

Coated graphite electrode: A pure graphite rod of 5 mm diameter was insulated by tight polyethylene tube, leaving 2.0 cm at one end for coating and 1.0 cm at the other end for connection. The polished electrode surface was coated with the active membrane by dipping the exposed end into the coating solution. The prepared electrode was precondition by soaking for 24 hrs in $1x10^{-3}$ mol L^{-1} CLP solution.

2.6. Electrode calibration

Ten mL aliquots of 1.0×10^{-7}-1.0×10^{-2} mol L^{-1} CLP standard solutions were transferred into 50 mL beaker and the sensor in conjunction with Ag/AgCl reference electrode was immersed in solution. The measured potential was plotted against the logarithm of CLP concentration. The electrode was washed with distilled water and dried with tissue paper between measurements.

2.7. Effect of soaking

The investigated electrode was soaked in 1.0 $x10^{-3}$ mol L^{-1} of the CLP solution at room temperature. A calibration graph was constructed for electrodes I, II and III, after time intervals covering the range 24 hrs to 25, 3o and 40 days respectively. The measurements were stopped when the slope of the calibration graph deviated largely from the Nernstian value and the electrode become out of use.

2.8. Effect of pH

The effect of pH of the test solution on the potential values of the electrodes system in solutions of different concentrations ($1x10^{-4}$, $1x10^{-3}$, $1x10^{-2}$ mol L^{-1}) of CLP solution was investigated. Aliquots of the investigated drug (50 mL) were transferred to 100-mL beakers and the tested ion- selective electrode in conjugation with Ag/AgCl reference electrode and a combined glass electrode were immersed in the same solution and the potential reading corresponding to different pH values were recorded.

2.9. Selectivity of electrodes

Selectivity coefficients $K^{pot}_{CLP,J^{z+}}$ were determined by the separate solution method, in which the following equation was, applied [30].

$$\text{Log } K^{Pot}_{CLP,J^{z+}} = (E_2-E_1)/S + \text{Log [CLP]} - \text{Log [} J^{z+}]^{1/z}$$

Where K^{pot} is the selectivity coefficient, E_1 is the electrode potential in 1×10^{-3} mol L^{-1} CLP solution. E_2 is the potential of the electrode in 1×10^{-3} mol L^{-1} solution of the interferent ion J^{z+} and S is the slope of the calibration plot. The selectivity of the electrodes towards sugars, amino acids, certain cations was studied.

2.10. Standard addition method

The electrode was immersed into a sample of known volume with unknown concentration and the equilibrium of E_1 was recorded. Then small volume of known concentration of standard drug solution was added into the testing solution and the equilibrium potential of E_2 was obtained. The concentration of the testing sample was calculated From the change of ΔE (E_2-E_1) [31].

3. Analytical applications

3.1. Applications to pharmaceutical formulations

3.1.1. Plavix® tablets

Ten tablets of plavix® (75mg / tablet) were finely powdered and accurate weight equivalent to (0.14 g) was used to prepare 1.0 ×10^{-2} mol L^{-1} using distilled water. Serial dilutions were done to obtain different concentrations in the range of (1 $x10^{-6}$-$1x10^{-3}$ mol L^{-1}) of CLP for the calibration method and ($1x10^{-5}$-$1x10^{-3}$, $1x10^{-6}$-$1x10^{-2}$ and $1x10^{-6}$-$1x10^{-4}$ mol L^{-1}) for standard addition method, respectively for plastic membrane, coated wire coated graphite electrodes.

3.1.2. Content uniformity assay of Plavix® tablets

Ten individual tablets of Plavix® (75 mg/tablet) were placed in separate 100-mL measuring flasks and dissolved in 100 mL distilled water pH 1, Each electrode was directly immersed into 50 mL of sample solution for three times and then washed with distilled water to reach steady potential between the individual measurements. The mean potential was used to evaluate the content uniformity from the calibration graph.

3.2. Applications to biological fluids (serum and urine)

0.1 N acetate buffer was added to serum solution dropwise until the suitable pH obtained. 1.0 mL aliquots of serum were transferred into a series of centrifugation tubes. Aliquots of standard aqueous solution of CLP were added so that the final concentration is in the range of 1.0×10^{-7}-1.0×10^{-2} mol L^{-1}. The tubes were mixed well 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 distilled water. While 1 mL aliquots of urine were transferred into a series of 100-mL measuring flasks. Aliquots of standard aqueous solution of CLP were added so that the final concentration is in the range of 1.0×10^{-6}-1.0×10^{-2} mol L^{-1}. The flasks were mixed well and completed to volume using distilled water. These solutions were analyzed as described above under electrode calibration and standard addition methods.

4. Results and Discussion

4.1. Nature and response characteristics of the electrodes

The critical response characteristics of plastic membrane, coated wire and coated graphite electrodes were determined and results were summarized in Table 1. The electrode (s) exhibits a Nernstian response over concentration range from 1.0 $x10^{-7}$-1.0 x 10^{-2} mol L^{-1} CLP for electrodes, with slopes of 55.97±0.460, 57.57±0.227 and 58.03±0.150 mV $daecade^{-1}$ for electrodes I, II and III, respectively as in Figure 2. Also they showed a fast and dynamic response of 20, 25 and 15s for a period of 25, 30 and 40 days for electrodes I, II and III, respectively, without significant change in electrode parameters.

4.2. Effect of soaking

The preconditioning process was carried out by soaking the electrodes in 1.0 x 10^{-3} mol L^{-1} CLP solution and calibration graphs were plotted after 24hrs. The slopes of calibration curves were 55.97±0.460, 57.57±0 and 58.03±0.150 mV $decade^{-1}$, at 25°C for electrodes I, II and III, respectively. The continuous soaking in the same solution causes the calibration plot slopes decreased slightly to 54.1mV $decade^{-1}$ after 10 days, and 52,3 mV $decade^{-1}$ after 20 days, then dropped to 45.4 mV $decade^{-1}$ after 25 days for the electrode I. Also the slopes for electrode II, decreased slightly with soaking to 54.3mV $decade^{-1}$ after 15 days, and continued to decrease reaching 53.8 mV $decade^{-1}$ after 20 days, then dropped to 49.0 mV $decade^{-1}$ after 30 days. Also the continuous soaking the electrode III, the calibration plot slope decreased to 56.5mV $decade^{-1}$ after 20 days, then dropped to 46.6 mV $decade^{-1}$ after 40 days. Figure 3 shows the calibration graphs for plastic membrane I, electrode after soaking.

4.3. Regeneration of the electrodes

The above discussion revealed that soaking of the electrodes in the drug solution for a long time has a negative effect on the response of the membrane towards CLP. The same effect appears after working with the electrode for a long time. The regeneration of the electrode was tried simply by reformation of the ion-exchange on the external gel layer of membrane. The regeneration of the CLP membrane was successfully achieved by soaking the exhausted electrodes for 24 hrs in a solution that was 1.0 $x10^{-2}$ mol L^{-1} of (PMA), (ARS) and (PTA), followed by soaking for 3 h in $1.0x10^{-2}$ mol L^{-1} CLP solution. Figure 4 show the calibration graphs for the exhausted electrode (slopes 45.00 mV $decade^{-1}$) for plastic membrane electrode I, and for the same electrode after regeneration (slopes 55.25mV $decad^{-1}$). It was found that the lifespan of the generated electrode (s) is limited to ≤ 6 h due ease of leaching of the lipophilic salts from the gel layer.

4.4. Effect of pH

The effect of pH of the CLP solutions using $1x10^{-3}$ mol L^{-1} drug solution on the electrode (s) potential were investigated. The solutions were acidified by the addition of very small volumes of 0.1 mol L^{-1} HCL then the pH value was increasing gradually using 0.1 mol L^{-1} NaOH. The potential for each pH value was recorded and then the potential-pH curves for CLP concentration were constructed as shown in Figure 5. It is obvious that the electrode(s) potential is practically independent in the pH range (1.2-4.6), and in this range the electrode can be safely used for CLP determination. Below pH 1.2, the potential of the electrode increased with increase of analyte acidity which may be ascribed to extraction of H^+ ions by membrane. While at pH more than 4.6, the response of the electrode decreased which may be attributed to increase of OH^- concentration [32].

4.5. Selectivity of the electrodes

The potentiometric selectivity coefficients of the prepared electrodes were investigated by separate solution method using inorganic cations, sugar and amino acids. The obtained results in Table 2, showed high selectivity toward CLP. The inorganic cations did not interfere because of differences in ionic size,

mobility and permeability. Also the electrodes exhibit high selectivity toward sugar and amino acids may be attributed to the difference in polarity and lipophilic nature their molecules relative CLP cations.

4.6. Quantification of clopidogrel bisulphate

The direct potentiometric determination of CLP in pure form using the proposed electrodes gave mean recoveries % of (99.59±0.213, 99.61±0.144 and 99.66±0.325 mV decad^{-1}) and (99.40±0.310, 99.28±0.414 and 99.61±0.417 mV decade^{-1}) for the three electrodes by calibration and standard addition methods respectively. The results obtained were compared with those of the UV spectrophotometric reference method [8]. No significant difference between two methods was observed with respect to precision and accuracy (Table 3).

.H$_2$SO$_4$

Figure 1. Chemical structure of clopidogrel bisulphate

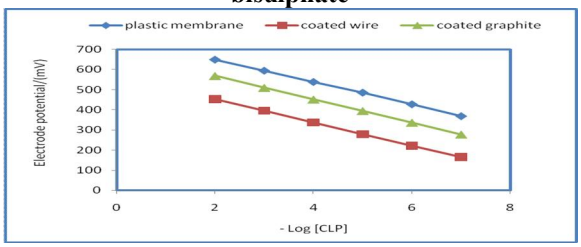

Figure 2. Typical calibration graphs of CLP electrodes

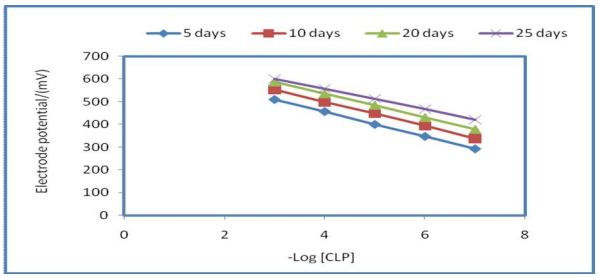

Figure 3. Calibration graphs obtained at 25±1°C after soaking the(CLP-PMA) plastic membrane electrode

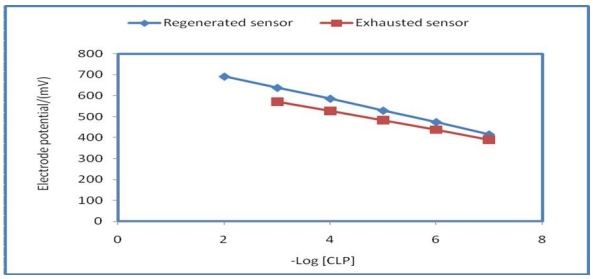

Figure 4. Regeneration of (CLP-PMA) plastic membrane electrode

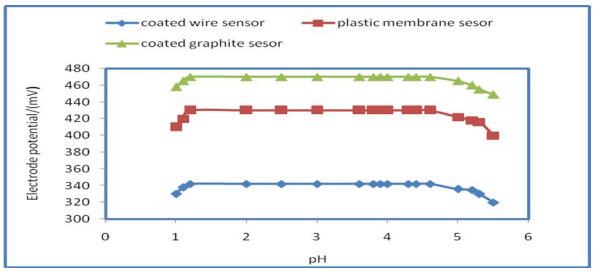

Figure 5. Effect of pH on the CLP electrodes potential

Table 1: Critical response characteristics of CLP electrodes

Parameter[a]	Plastic membrane electrode	Coated wire electrode	Coated graphite electrode
Slope(mV decade^{-1})	55.97±0.460	57.57±0.227	58.03±0.150
Intercept	763.64	667.90	684.60
Correlation coefficient (r)	0.9998	0.9999	0.9999
Linear range (mol L^{-1})	1x10^{-7}-1x10^{-2}	1x10^{-7}-1x10^{-2}	1x10^{-7}-1x10^{-2}
Detection limit (mol L^{-1})	5.01x 10^{-8}	4.1x10^{-8}	5.0x10^{-8}
Response time for10^{-3} mol L^{-1}(s)	20	25	15
Working pH range	1.2-4.6	1.2-4.6	1.2-4.6
Life time/day	25	30	40
Accuracy (%)	99.09±0.656	99.72±0.243	99.28±0.857
Standard deviation (%)	0.460	0.227	0.150
Robustness[b]	99.48±0.537	99.36±0.337	99.51±0.445
Ruggedness[c]	99.51±0.430	99.26±0.650	98.77±0.199

[a] Mean of six measurements

[b] A small variation in method parameters were studies using acetate buffer pH 4±0.5

[c] Comparing the results by those obtained by different electrodes assemblies using (Jenway 3510)pH-meter

Table 2: Selectivity coefficients (K^{pot}) for the CLP electrodes

	K^{pot}		
Interferent	Plastic membrane electrode	Coated wire electrode	Coated graphite electrode
Ni^{2+}	2.9×10^{-5}	1.2×10^{-5}	6.9×10^{-5}
Sn^{2+}	1.0×10^{-5}	2.9×10^{-4}	1.2×10^{-4}
Ba^{3+}	1.9×10^{-4}	2.4×10^{-4}	1.1×10^{-4}
Ca^{2+}	4.6×10^{-4}	1.9×10^{-4}	8.5×10^{-4}
Cd^{2+}	1.3×10^{-4}	1.6×10^{-5}	3.2×10^{-5}
Zn^{2+}	1.4×10^{-4}	4.9×10^{-5}	3.6×10^{-5}
Mg^{2+}	7.8×10^{-5}	7.3×10^{-5}	5.3×10^{-5}
K^{+}	7.2×10^{-5}	1.7×10^{-5}	2.9×10^{-5}
Na^{+}	1.3×10^{-4}	3.0×10^{-5}	3.4×10^{-5}
NH_4^{+}	3.0×10^{-4}	1.9×10^{-5}	1.4×10^{-4}
Talc	1.0×10^{-4}	1.0×10^{-4}	2.3×10^{-4}
Thymine	4.9×10^{-3}	6.6×10^{-4}	6.5×10^{-4}
Thymidine	3.1×10^{-4}	8.7×10^{-4}	5.5×10^{-4}
Ornithine	8.5×10^{-4}	3.5×10^{-4}	2.7×10^{-4}
Glycine	1.8×10^{-3}	4.6×10^{-4}	3.6×10^{-4}
L-Histidine	6.6×10^{-4}	8.0×10^{-4}	7.3×10^{-4}
L-Glutamine	1.2×10^{-3}	4.1×10^{-4}	3.2×10^{-4}
L-Cystine	1.6×10^{-3}	5.6×10^{-4}	4.5×10^{-4}
Starch	3.7×10^{-4}	7.3×10^{-5}	1.8×10^{-4}
Glucose	6.1×10^{-6}	1.2×10^{-4}	1.6×10^{-4}

Table 3: Statistical treatment of the data obtained for the determination of CLP in pure form by proposed and reference methods

Statistical parameter	Reference method [8]	Plastic membrane electrode		Coated wire electrode		Coated graphite electrode	
		Calibration method	Standard addition method	Calibration method	Standard addition method	Calibration method	Standard addition method
Mean	99.45	99.59	99.40	99.61	99.28	99.66	99.61
n	6	7	5	7	5	7	6
Variance	0.060	0.045	0.096	0.021	0.171	0.106	0.174
SD	0.245	0.213	0.310	0.144	0.414	0.325	0.417
SE**	0.100	0.080	0.126	0.054	0.185	0.123	0.170
% RSD	0.246	0.214	0.312	0.145	0.417	0.326	0.418
t-test		1.09(2.20)*	0.30(2.28)*	1.41(2.20)*	0.81(2.26)*	1.32(2.20)*	0.81(2.23)*
F-test		1.33(4.39)*	1.60(5.05)*	3.00(4.39)*	2.85(5.19)*	1.76(4.95)*	2.90(5.05)*

*The figures between parentheses are the theoretical values of t- and F- tests at P=0.05 [33], % SE** =%RSD / $\sqrt{n}$

Table 4: Precision data for CLP using proposed method

Parameters	Plastic membrane electrode	Coated wire electrode	Coated graphite electrode
Intra-day			
Mean	99.53	99.08	99.61
SD	0.46	0.520	0.535
SE	0.27	0.300	0.308
% RSD	0.462	0.524	0.537
Inter-day			
Mean	99.02	99.44	99.72
SD	0.535	0.497	0.254
SE	0.31	0.268	0.146
% RSD	0.541	0.499	0.255

Intra-day: within the day
Inter-day: consecutive days

Table 5 : Statistical treatment of the data obtained for the determination of CLP in Plavix® tablets by proposed and reference methods

Statistical parameter	Reference Method [8]	Plastic membrane electrode		Coated wire electrode		Coated graphite electrode	
		Calibration method	Standard addition method	Calibration method	Standard addition method	Calibration method	Standard addition method
Mean	99.64	99.35	99.59	99.20	99.43	99.48	99.78
n	6	7	5	7	8	7	6
Variance	0.094	0.070	0.141	0.388	0.386	0.169	0.100
SD	0.306	0.267	0.375	0.623	0.621	0.411	0.310
SE**	0.125	0.100	0.167	0.235	0.219	0.155	0.130
% RSD	0.307	0.268	0.377	0.628	0.634	0.413	0.311
t-test		1.76(2.20)*	0.23(2.26)*	1.61(2.20)*	0.82(2.18)*	0.78(2.20)*	0.76(2.23)*
F-test		1.28(4.39)*	1.55(5.19)*	4.13(4.95)*	4.04(4.88)*	1.88(4.95)*	1.11(5.05)*

*The figures between parentheses are the theoretical values of t- and F- tests at P=0.05 [33], % SE** = %RSD / $\sqrt{n}$

Table 6: Determination of CLP in urine and serum by the proposed electrodes

Statistical parameter	Plastic membrane electrode		Coated wire Electrode		Coated graphite electrode	
	Urine solution	Serum solution	Urine Solution	Serum solution	Urine solution	Serum solution
Mean±SD	99.39±0.254	99.19±0.387	99.48±393	99.62±0.422	99.26±0.655	99.10±0.543
n	7	7	6	6	6	6
Variance	0.065	0.150	0.154	0.178	0.429	0.295
SE**	0.096	0.056	0.160	0.172	0.267	0.222
% RSD	0.256	0.391	0.395	0.424	0.660	0.548

5. Validation of the proposed method

5.1. Linearity and detection limit (LOD)

Under the optimal experimental ISE conditions, a linear relationship exists between the electrode potential /mV and the logarithm of corresponding concentration of the investigated drug. And the values of LOD were indicating that the proposed method is sensitive for detection of very small concentrations of CLP. The regression data, correlation coefficient (r) and other statistical parameters were listed in Table1.

5.2. Robustness and ruggedness

The robustness of the proposed ISE method was tested by using acetate buffer pH 4±0.5 and the percentage recoveries were 99.48±0.537, 99.36±0.337 and 99.51±0.445 mV decad^{-1} for the three electrodes, these results were closely in agreement with those obtained from standard drug solution, (Table1). The reproducibility upon using another model of pH-meter (Jenway,3510) was indicated by the results obtained as percentage recoveries were 99.51±0.430, 99.26±0.650 and 98.77±0.199 for the same electrodes.

5.3. Accuracy

The accuracy of the proposed ISE method was investigated by the determination of CLP in its pharmaceutical preparations without interfering from the coformulated adjuvants as indicated by the mean recoveries value of (99.09±0.658, 99.72±0.243 and 99.28±0.857 mV decad^{-1}) for electrodes I, II and III, respectively.

5.4. Precision

The precision of the ISE method measured as percentage relative standard deviation (% RDS) was tested by repeating the proposed ISE method for analysis of the investigated CLP in intra-day and inter-day to nine replicates. The obtained %RSD values were (0.462%, o.541%), (0.524%, 0.499%) and (0.447%, 0.201%) for the three electrodes. The % RSD values are less than 2%, indicating good precision (Table 4).

6. Analytical applications

6.1. Applications to pharmaceutical formulations

6.1.1. Plavix® tablets

In order to evaluated analytical usefulness of the proposed ISE potentiometric methods, CLP was determined in Plavix® tablets using calibration and standard addition methods. Table 5, show comparison of the proposed and the reference methods for determination of CLP in tablet.

6.1.2. 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 the mean recoveries ± standard deviations were 99.91±0.671, 99.40±0.657 and 99.14±0.446 for plastic membrane, coated wire and coated graphite electrodes respectively.

6.2. Application to serum and urine

The proposed ISE method was successfully applied to determine CLP in biological fluids as

human serum and urine. The results obtained were summarized in Table 6.

7. Conclusion

The proposed potentiometric methods based on the construction of different types of selective electrodes with ion exchangers might be useful analytical characteristics for the determination of CLP in pure form, pharmaceutical formulations 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 and inexpensive making it an excellent tool for the routine determination of CLP in quality control laboratories.

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