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ELECTROCHEMICAL STUDIES OF MODIFIED 2-HYDROXYPROPYL BETA CYCLODEXTRIN AND MODIFIED CARBON NANOTUBES SENSORS FOR DETERMINATION OF ANTI-DIABETIC PIOGLITAZONE HYDROCHLORIDE

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ABSTRACT

The present study aimed to suggest a simple, accurate and sensitive electrochemical method for the potentiometric determination of pioglitazone hydrochloride (PLZ). The developed method was conducted by the incorporation of PLZ with sodium tetraphenyl borate (TPB) to fabricate four PLZ-TPB sensors using *o*-nitrophenyloctyl ether (*o*-NPOE) as a plasticizer in a polymeric matrix polyvinyl chloride (PVC). Sensor I and sensor II were conventional coated wire membrane type, while sensor II was modified using 2-hydroxypropyl beta cyclodextrin (β-CD) as ionophore. Sensor III and sensor IV were carbon paste type and sensor IV was modified using carbon nanotubes (CNTs). Under optimum condition the proposed sensors displayed Nernstian responses of 53.8±0.8 and 55.5±0.6, 57.1±0.4 and 57.7±0.1 mV decade^{-1} for sensors I, II, III and IV, respectively at 25˚C over drug concentration ranges of 1.0×10^{-6}-1.0×10^{-3} and 1.0×10^{-6}-1.0×10^{-2} mol L^{-1}, 1.0×10^{-7}-1.0×10^{-2} and 1.0×10^{-8}-1.0×10^{-2} mol L^{-1} with lower detection limits of 5.0×10^{-7}, 4.7×10^{-7} and 5.0×10^{-8} and 4.8×10^{-9} mol L^{-1} for the four mentioned sensors, respectively. The selectivity of the proposed method towards the investigated drug and some possible interfering species was studied using a separate solution method. The effect of pharmaceutical additives and some related pharmacological

action drugs was also tested. The developed method was validated and gave excellent results for the determination of PLZ in its pharmaceutical formulations and biological fluids.

KEYWORDS: Pioglitazone hydrochloride; Carbon paste; carbon nanotubes sensor; 2-hydroxypropyl beta-cyclodextrin; Pharmaceutical dosage forms; Biological fluids.

1. INTRODUCTION

The use of nanoparticles has explored as a new class of chemical read-outs for assaying a variety of chemical and biological species because of their unique physico- chemical and size dependent properties. Promising applications have been found by carbon nanotubes (CNTs) in various electrochemical sensors, diverse chemicals of food quality, clinical and biological interest.[1] Various advantages of CNTs as sensor materials have been shown. These advantages can be attributed to the high thermal conductivity, remarkable mechanical properties, their chemical stability and high surface to volume ratio of CNTs.[2]

Cyclodextrins are a group of structurally related natural product formed during bacterial digestion of cellulose. They are a family of cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity. As a result of their molecular structure and shape, they possess a unique ability to act as molecular containers by entrapping guest molecules in their internal cavity.[3] Water-soluble cyclodextrin derivatives of commercial interest included the hydroxypropyl derivatives (Figure 1a) were used in sensor modifications.[4] Cyclodextrins play an important role to form accommodation for a wide variety of organic, inorganic and biologic guest molecules stable host-guest inclusion complexes. They have been previously applied as sensor ionophores for potentiometric determinations.[5]

Pioglitazone hydrochloride (PLZ) is a member of the family of medications known as thiazolidinediones. It is used to lower high blood sugar associated with type 2 diabetes (Figure 1b). Thiazolidinediones such as PLZ help insulin to work more effectively. By keeping blood sugar at a desired level, PLZ can help to prevent or delay the long-term problems associated with uncontrolled high blood sugar, such as kidney disease, eye problems, nerve problems, heart disease. PLZ may be used alone when blood sugar is not only controlled by diet and exercise or in combination with one other diabetes medication, including metformin and gliclazide.[6]

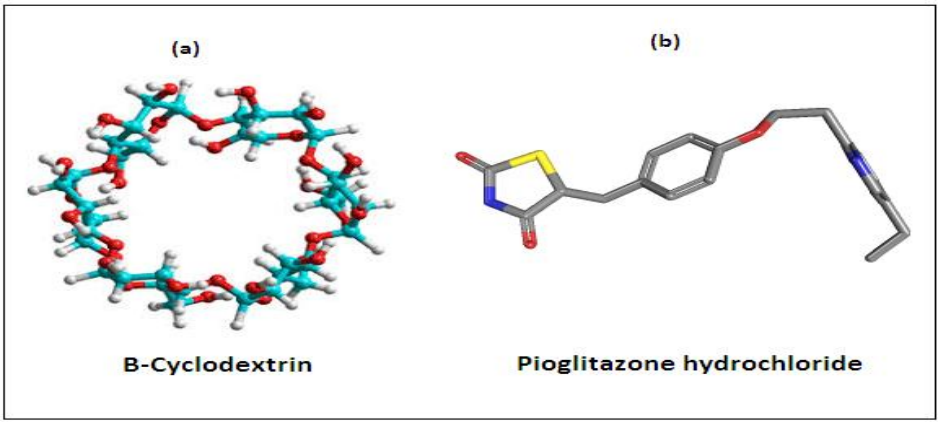

Figure 1: Chemical structures of (a) β-Cyclodextrin and (b) Pioglitazone hydrochloride.

The literature survey addressed several methods have been reported for the determination of PLZ including, high performance liquid chromatography,[7-10] high performance liquid chromatography coupled with mass spectrometry,[11-14] thin layer chromatography,[15-17] spectrophotometry,[18-20] spectrofluorimetry,[21,22] chemiluminescence,[23] electrochemical methods such as voltammetry[24,25] and potentiometry.[26]

The aim of the present study is to develop simple and sensitive four types of PLZ sensors. Coated wire membrane type sensor I and modified β-CD sensor II, carbon paste sensor III and modified carbon nanotubes carbon paste sensor IV using PLZ-TPB as electroactive material. The fabricated sensors were used for determination of PLZ in bulk drug, its pharmaceutical formulations and biological fluids. The proposed method was validated according to ICH guidelines.[27]

2. EXPERIMENTAL

2.1. Materials and reagents

Sodium tetraphenyl borate (TPB) 99.9%, high purity graphite powder (1-2 µm), high molecular weight of poly vinyl chloride (PVC) and multi-wall carbon nanotubes powder (carbon > 95.0%, O.D. x L 6-9 nm x 5 µm), 2-hydroxypropyl β-cyclodextrin (β-CD) were purchased from Sigma-Aldrich, Hamburg, Germany. Di-octylphthalate (DOP) 99.5%, di-butyl phthalate (DBP) 99.0%, di-butyl sebacate (DBS) ≥ 97.0%, di-octyl sebacate (DOS) ≥ 97.0%, *o*-nitrophenyloctyl ether (*o*-NPOE) 99.0%, and tetrahydrofuran (THF) were provided by Fluka, Switzerland. Sodium hydroxide 98.0%, Zinc sulphate≥ 99.0% and hydrochloric acid 36.5% were purchased from BDH laboratory supplies, Poole, UK. Pure grade pioglitazone hydrochloride was kindly supplied by HI Pharm Co., El-Bour, Egypt. The pharmaceutical preparation (Glitazone®Tablets) each tablet was claimed to contain 15 mg

mL^{-1} purchased from local drug stores. Phosphate buffer of pH 6.0 was prepared by mixing 13.2 mL of 1.0 mol L^{-1} K_2HPO_4 (dibasic) with 86.8 mL of 1.0 mol L^{-1} KH_2PO_4 (monobasic) and the pH was adjusted to the exact value of pH 6.0. Urine samples were obtained from healthy volunteers and serum samples (Multi-Serum Normal, Randox Laboratories, UK) were obtained from commercial sources. All chemicals used throughout the experimental analysis were of analytical grade. Distilled water was used throughout all measurements.

2.2. Instrumentation

The potentiometric and pH measurements were carried out using HANNA instrument model-211 microprocessor pH-meter (Romania). Ag/AgCl electrode was used as an external reference electrode. AREX heating magnetic stirrer connected with a circulator thermostat was used to control the temperature of the test solutions. Ivyman distiller system –AC-L8 was used for distilled water. Scanning electron microscope (SEM), JEOL JSM-6060 LV-(Japan) was used for surface structure studies of carbon nanotubes sensor.

2.3. Preparation of analytical solutions

2.3.1. Standard drug solution

A standard solution of 1.0×10^{-1} mol L^{-1} PLZ was freshly prepared daily by dissolving 3.929 g in 100 mL distilled water. Serial dilutions using distilled water were carried out to obtain working solutions in the range of (1.0×10^{-8}-1.0×10^{-1} mol L^{-1}).

2.3.2. Preparation of pioglitazone hydrochloride tablet solution

Not less than 20 tablets (Glitazone® tablet) each tablet claimed to contain 15 mg pioglitazone hydrochloride, were finely powdered and an accurate amount equivalent to prepare 1.0×10^{-2} mol L^{-1} was transferred into the 50-mL volumetric flask, and mixed with 10 mL methanol. The solution was centrifuged, followed by filtration. The obtained clear solution was completed with distilled water to the mark. A working solution was prepared by serial dilutions using a distilled water to obtain the concentration ranges of 1.0×10^{-6}-1.0×10^{-3} and 1.0×10^{-6}-1.0×10^{-2} mol L^{-1} for sensors I and II, respectively, 1.0×10^{-7}-1.0×10^{-2} and 1.0×10^{-8}-1.0×10^{-2} mol L^{-1} for sensors III and IV, respectively.

2.3.3. Preparation of spiked serum and urine solutions

In order to determine the PLZ in human serum and urine, spiking technique method was employed. Firstly, the human serum and urine samples were adjusted at pH 6 using phosphate buffer. Approximately, 1.0 mL of the previously adjusted human serum or 5.0 mL of urine

solutions was transferred to centrifugation tubes and spiked with accurately measured aliquots of PLZ solutions. Then vortex was done in 5.0 min. For human serum 1.0 mL acetonitrile, 0.1 mL of 0.1 mol L^{-1} NaOH, followed by 1.0 ml of $ZnSO_4.7\ H_2O$ (5.0% w/v) were added, where most of the interfering species (mainly proteins) were removed by precipitation.[28] After centrifugation for 30 min at 2500 rpm, the clear supernatant layer was filtered through 0.5 µm Milli-pore filter. For human urine no further treatment was done and working solutions were then prepared by serial dilutions to obtain PLZ concentration ranges of 1.0×10^{-5}-1.0×10^{-3} and 1.0×10^{-6}-1.0×10^{-3} mol L^{-1} for sensors I and II, respectively, 1.0×10^{-7}-1.0×10^{-3} and 1.0×10^{-8}-1.0×10^{-3} mol L^{-1} for sensors III and IV, respectively. The prepared solutions were subjected to analysis using PLZ-TBP fabricated sensors and the % recoveries for each concentration was determined and calculated using the calibration graph of the standard drug solution.

2.4. Preparation of pioglitazone-tetraphenylborate ion pairs

The electroactive material PLZ-TPB of the developed sensors was prepared by the incorporation of 50 mL of 1.0×10^{-2} mol L^{-1} of PLZ solution with 50 mL of 1.0×10^{-2} mol L^{-1} of TPB. The obtained precipitate was left overnight and filtered using a Whatman filter paper No.2, washed with distilled water, dried at room temperature for 24 h and ground to a fine powder. Elemental analysis was performed to confirm the composition of the ion pair. The elemental data was confirmed that the ion pair formed was $[(C_6H_5)_4B][C_{19}H_{20}N_2O_3S]$ which revealed that the composition was found to be 1:1 for PLZ:TPB. The calculated percentages of C, H, O, N and S were found to be 76.41%, 5.97%, 7.11 %, 4.15 % and 4.75%, respectively. While the found percentages were 76.22%, 5.83%, 7.13%, 4.11 and 4.63% for C, H, O, N and S, respectively.

2.5. Preparation of forced degradation products of pioglitazone hydrochloride

The forced degradation of PLZ was carried out by adding 1.0 mL of 1.0 mol L^{-1} hydrochloric acid or 1.0 mol L^{-1} sodium hydroxide or/and 5.0 % hydrogen peroxide to about 10 mg of PLZ. The samples were refluxed separately at 80 ˚C for 12 h. The fabricated sensors were used for determination of the investigated drug in its forced degradation products. The solution was then cooled and neutralized then diluted with methanol. The degradation process completeness was tested on silica gel 60 F_{254} using KH_2PO_4 pH (3.5): methanol (55:45 v/v) as mobile phase. The degradation product was filtered, washed and then left to dry in the air for 3 days.

2.6. Sensor construction

2.6.1. Preparation of coated membrane and modified β-CD sensors

The conventional coated membrane of PLZ was prepared by mixing 190 mg polyvinyl chloride PVC with 10.0 mg of PLZ-TPB ion pair and 0.35 mL *o*-NPOE as plasticizer. The electroactive material PLZ-TPB was dissolved in 5.0 mL tetrahydrofuran (THF) as solvent mediator. The top of Al wire was coated with the membrane and let to dry in air overnight. The modified β-CD membrane was prepared by adding 50 mg of 2-hydroxypropyl β-CD to the above contents and the previously mentioned procedure was followed.

The constructed sensors were preconditioned by soaking each sensor in 1.0×10^{-3} mol L^{-1} PLZ for 12 h and stored in the same solution. All potentiometric measurements were performed using the following cell assembly: Al wire coated membrane/test solution/Ag/AgCl.

2.6.2. Preparation of carbon paste and modified carbon nanotubes sensors

In an agate mortar about 60 % of pure graphite powder (1-2 μm) was mixed with 30.0 % *o*-NPOE as liquid plasticizer and 10.0 % ion pair (PLZ-TPB) to fabricate the carbon paste. Homogenous paste was obtained by mixing the above mixture well and Teflon holder (3.0 mm in diameter) was used for packing the paste carefully. Electrical contact was made with a copper rod through the center of the sensor holder. Filter paper was used for surface polishing to obtain shiny, smooth and reproducible surface. The sensor was conditioned by dipping it in 1.0×10^{-3} mol L^{-1} PLZ solution for 8 h. All potentiometric measurements were performed using the following cell assembly: carbon paste/test solution/Ag/AgCl reference electrode.

The modification of carbon paste was carried out by adding a small amount of multi-wall carbon nanotubes particles and the paste was homogeneously mixed. The fabricated paste was carefully packed in sensor holder and was left to dry for one day. The shiny and smooth surface was obtained by polishing the sensor surface using filter paper. The sensor was conditioned by dipping it in 1.0×10^{-3} mol L^{-1} PLZ solution for 8 h. All potentiometric measurements were performed using the following cell assembly: modified carbon nanotubes/test solution /Ag/AgCl reference electrode. The surface structure of carbon paste sensors was examined using scanning electron microscope (SEM) as shown in Figures 2a and 2b.

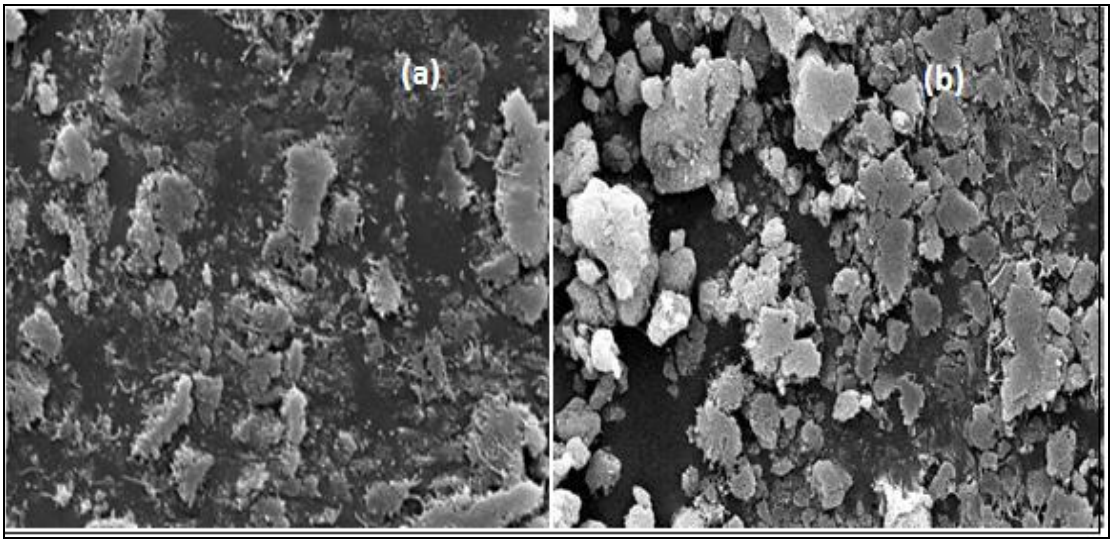

Figure 2: Scanning electron images of the carbon paste sensor surface (a) carbon paste, (b) modified carbon nanotubes (MCNTs) carbon paste.

2.7. Sensor calibration

In order to study the calibration graphs of the developed PLZ-TPB sensors, working solutions containing 1.0×10^{-8}-1.0×10^{-1} mol L^{-1} of PLZ were employed. The calibration graphs were plotted for all potentiometric measurements using the fabricated sensors in conjunction with Ag/AgCl reference electrode. The potential of each sensor was recorded and plotted against the logarithm of drug concentration.

2.8. Standard addition method

The determination of PLZ in its pharmaceutical formulations was carried out using standard addition method. The method was based on adding small increments of the investigated drug test solution vs. the sensor potential. Each fabricated sensor was immersed into 50 mL drug test solution with unknown concentration and the equilibrium potential of E_1 was recorded. Then 0.1 mL of the standard drug solution was added into the testing solution and the equilibrium potential E_2 was recorded. The concentration of the testing sample can be obtained from the change of potential ($\Delta E = E_2 - E_1$).

3. RESULTS AND DISCUSSION

3.1. Nature and response characteristics of PLZ sensors

PLZ sensors were fabricated using PLZ-TPB as electroactive material, which is water insoluble but soluble in organic solvent such as tetrahydrofuran (THF). The performance characteristics of the fabricated sensors were studied (Figure 3).

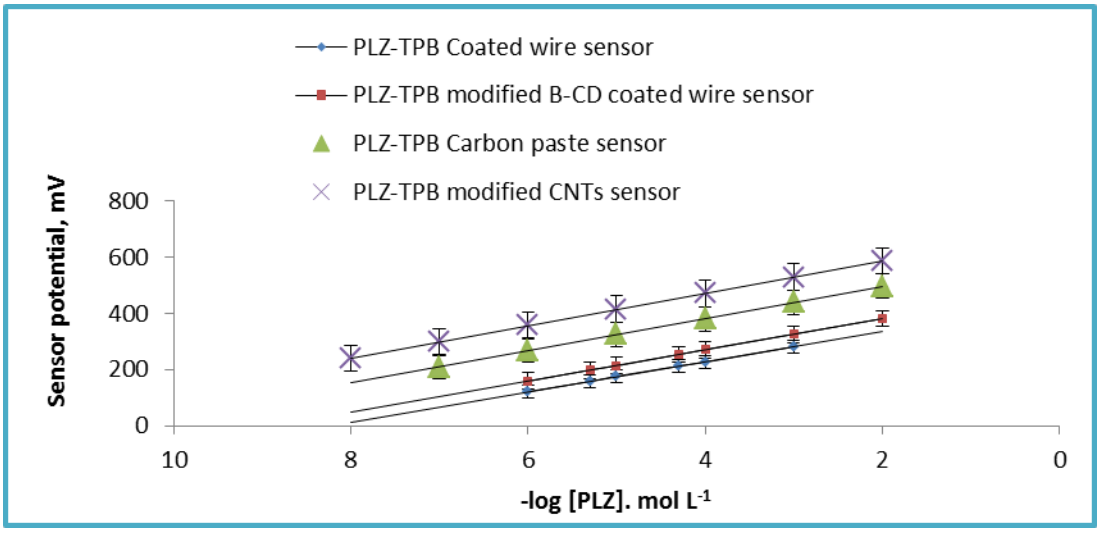

Figure 3: Typical calibration graphs of PLZ-TPB coated wire membrane and modified 2- hydroxypropyl β-CD, PLZ-TPB carbon paste and modified CNTs sensors.

It was found that PLZ-TPB sensors were displayed Nernstian responses (53.8±0.8, 55.5±0.6, 57.1±0.4 and 57.7±0.1 mV decade^{-1}) at 25˚C over the drug concentration ranges 1.0×10^{-6}-1.0×10^{-3}, 1.0×10^{-6}-1.0×10^{-2}, 1.0×10^{-7}-1.0×10^{-2} and 1.0×10^{-8}-1.0×10^{-2} mol L^{-1} with lower detection limits of 5.0×10^{-7}, 4.7×10^{-7}, 5.0×10^{-8} and 4.8×10^{-9} mol L^{-1} for sensors I, II, III and IV, respectively.

As presented in Table 1, the results clarified that the modified β-CD (sensor II) gave better performance characteristics than that of the conventional coated wire membrane (sensor I). This can be attributed to the high stability of the complex formed between the cationic drug and the chelating agent due to the presence of high OH group donation. Thus the selectivity and sensitivity of the membrane were enhanced.

The use of 2-hydroxypropyl β-CD as ionophore provided high stability of the complex formed between the cationic drug and the chelating agent which can be attributed to the presence of high donation (OH-groups), thus the membrane selectivity and sensitivity were substantially enhanced.[4]

Table 1: Electrochemical response characteristics of PLZ-TPB sensors.

Parameter[a]	PLZ-TPB Coated wire membrane (sensor I)	PLZ-TPB Modified β-CD (sensor II)	PLZ-TPB Carbon paste (sensor III)	PLZ-TPB Modified CNTs (sensor IV)
Slope (mV decade^{-1})	53.8±0.8	55.5±0.6	57.1±0.4	57.7±0.1
Intercept	442.7	492.8	610.2	702.6
Correlation coefficient I	0.999	0.999	0.999	0.999
Linear range (mol L^{-1})	1.0×10^{-6}-1.0×10^{-3}	1.0×10^{-6}-1.0×10^{-2}	1.0×10^{-7}-1.0×10^{-2}	1.0×10^{-8}-1.0×10^{-2}
Lower limit of detection	5.0×10^{-7}	4.7×10^{-7}	5.0×10^{-8}	4.8×10^{-9}
Response time/s	40	30	20	15
Working pH range	3-8	3-8	3-8	3-8
Life time/day	50	60	45	70
Temperature ˚C	25˚C	25˚C	25˚C	25˚C
Accuracy (%)	98.7± 0.9	99.2± 0.4	99.5± 0.5	99.6± 0.3
Robustness[b]	98.9± 0.6	99.5± 0.5	99.7± 0.2	99.9± 0.1
Ruggedness[c]	98.6±0.5	99.2±0.1	99.4±0.7	99.8±0.4

[a]Mean of six measurements [b]A small variation in method parameters were carried out as pH of phosphate buffer (pH 8.0±1), [c]Comparing results with those obtained by different sensor assemblies using Jenway 3510 pH meter

In the case of carbon paste sensor III and MCNTs carbon paste sensor IV, it was found that the addition of 1.0-5.0 w% carbon nanotubes to modify carbon paste sensor played an important role in the enhancement of sensor response. The improvement effect of carbon nanotubes on the performance of the modified sensor can be attributed to their chemical stability and good electric conductivity properties of CNTs. Also, their porous surface structure and large surface area facilitate a better electrolyte, sensor interface that improves the wetting property with solvents.

3.2. Effect of plasticizers

The effect of plasticizers was carefully studied using five different kinds of plasticizers such as DOP with dielectric constant (ε = 5.1), DBP (ε = 6.4), DBS (ε = 4.5), DOS (ε = 4.0) and *o*-NPOE (ε =24.0). The use of each plasticizer content ratio 45.0, 48.0, 50.0, 55.0 and 60.0 w% was investigated. Table 2, showed the effect of plasticizers on the slopes of PLZ-TPB sensors. It was clear that the use of *o*-NPOE as plasticizer provided good performance characteristics of the sensors. This can be attributed to the higher dielectric constant (ε = 24.0) of *o*-NPOE than other plasticizers. From the obtained results it can be concluded that the use of plasticizers in the fabrication of sensors plays a very important role in the improvement of their performance characteristics. They give some permeable properties to the sensors and improve their mechanical stability. Also, in case of carbon paste sensors the selection of suitable plasticizer will improve the physical properties of these sensors and promote the binding between their carbon particles.

Table 2: Effect of plasticizers on the slopes of the fabricated PLZ-TPB sensors.

Slope (mV decade^{-1})				
Plasticizer	**PLZ-TPB Coated wire membrane (sensor I)**	**PLZ-TPB coated wire Modified β-CD (sensor II)**	**PLZ-TPB Carbon paste (sensor III)**	**PLZ-TPB Modified CNTs (sensor IV)**
DOS	49.9	48.3	52.4	53.2
DBS	50.4	51.7	52.9	55.4
DOP	51.0	53.2	54.9	55.9
DBP	51.6	55.0	56.5	56.6
0-NPOE	53.8*	55.5*	57.1*	57.7*

***The optimum value for the studied sensors**

3.3. Response time

The effect of response time of fabricated sensors was carefully investigated. The response time of sensors is the time required for sensors to reach a stable potential reading. The evaluation of response time of the fabricated sensors PLZ standard solutions in the range of 1.0×10^{-8}-1.0×10^{-1} mol L^{-1} were done. It was found that the dynamic response times for sensors I and II were 40 and 30 s while in the case of sensors III and IV the response times were 20 and 15 s for a period of 50, 60, 45 and 70 days for sensors I, II, III and IV, respectively, without significant change in the sensor parameters.

2.4. Effect of pH

The effect of pH on the potential of the fabricated sensors was studied using 1.0×10^{-3} mol L^{-1} PLZ solution. The test solution was firstly acidified using 0.1 mol L^{-1} hydrochloric acid and the potential readings were recorded, then the pH was gradually increased using 0.1 mol L^{-1} sodium hydroxide. The recorded potentials were plotted vs. pH. Figure 4 showed that the fabricated PLZ-TPB sensors displayed safe pH range at 3-8. It was noticed that below pH 3 the potential reading was decreased due to the high acidity and the interference of H^+ ion. While, at higher pH more than 8 the sensors displayed sharp decrease in potential due to the effect of OH^- on the test solution.

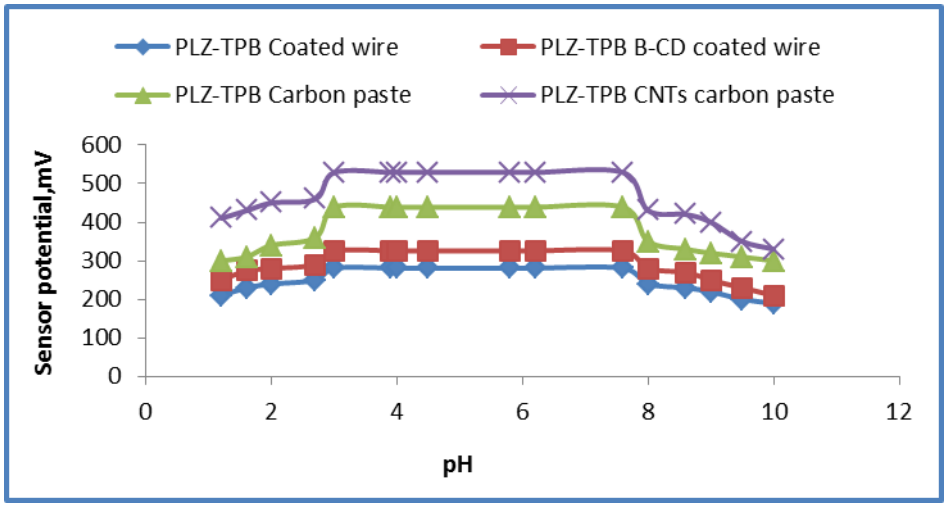

Figure 4: Effect of pH on PLZ-TPB sensors using (1.0 x10^{-3} mol L^{-1}) PLZ.

2.5. Selectivity of sensors

To study the selectivity coefficients of PLZ-TPB sensors, the selectivity of fabricated sensors was tested towards different common cations, sugars, amino acids and some additive substances using a separate solution method.[29] Also, the influence of some related pharmacological action drugs were examined. The following equation was applied to the selectivity coefficients of the proposed sensors.

$$\text{Log } K^{pot}_{PLZ} J^{z+} = (E_2 - E_1)/ S + \log [\text{PLZ}] - \log [J^{z+}]^{1/z}$$

Where, E_1 is the electrode potential in 1.0×10^{-3} mol L^{-1} PLZ solution, E_2 is the potential of the electrode in 1.0×10^{-3} Mol L^{-1} solution of the interfering ion J^{z+} and S is the slope of the calibration plot. The results reported in Table 3 clarified the high selectivity of the fabricated sensors. The main mechanism of selectivity is dependent on the matching between the locations of lipophilic sites in the two competing species in the bathing solution side and those present in the receptor of ion pair. The inorganic cations, sugars and some amino acids showed an insignificant interference effect during the determination of PLZ. Also, the effect of some related pharmacological action drugs such as metformin hydrochloride and gliclazide was examined. The obtained results showed no interference effect during the determination of PLZ.

Table 3: Selectivity coefficients ($K^{Pot}_{PLZ^+}$) for PLZ-TPB sensors using a separate solution method (1.0×10^{-3} mol L^{-1}PLZ).

Interferent	$K^{Pot}_{PLZ^+}$			
	PLZ-TPB Coated wire membrane (sensor I)	PLZ-TPB Modified β-CD (sensor II)	PLZ-TPB Carbon paste (sensor III)	PLZ-TPB Modified CNTs (sensor IV)
Na^+	1.6×10^{-3}	1.3×10^{-4}	3.6×10^{-4}	3.2×10^{-4}
K^+	5.5×10^{-3}	4.4×10^{-4}	1.9×10^{-4}	1.8×10^{-4}
NH_4^+	5.3×10^{-3}	6.5×10^{-4}	6.5×10^{-4}	6.7×10^{-4}
Ca^{2+}	8.8×10^{-3}	3.8×10^{-4}	5.2×10^{-4}	6.3×10^{-4}
Mg^{2+}	6.1×10^{-4}	2.4×10^{-4}	6.8×10^{-4}	8.8×10^{-4}
Zn^{2+}	5.0×10^{-4}	7.3×10^{-4}	5.5×10^{-4}	7.7×10^{-4}
Cu^{2+}	4.3×10^{-3}	6.6×10^{-3}	6.3×10^{-4}	6.5×10^{-4}
Fe^{3+}	2.3×10^{-4}	4.5×10^{-4}	1.2×10^{-4}	2.4×10^{-4}
Al^{3+}	8.7×10^{-3}	2.9×10^{-3}	9.4×10^{-4}	6.3×10^{-4}
Glucose	1.6×10^{-3}	1.7×10^{-3}	1.1×10^{-3}	5.4×10^{-4}
Lactose	6.0×10^{-3}	1.4×10^{-3}	7.0×10^{-3}	2.2×10^{-4}
Sucrose	2.3×10^{-5}	9.5×10^{-5}	2.4×10^{-5}	8.5×10^{-4}
Starch	5.6×10^{-4}	1.8×10^{-4}	5.5×10^{-4}	7.4×10^{-4}
Serine	6.5×10^{-3}	5.7×10^{-3}	1.4×10^{-3}	4.9×10^{-5}
Glycine	7.8×10^{-3}	3.4×10^{-3}	3.3×10^{-3}	2.0×10^{-5}
Histadine	6.3×10^{-4}	4.1×10^{-4}	6.1×10^{-4}	7.2×10^{-5}
Thymine	4.4×10^{-4}	1.2×10^{-4}	1.9×10^{-4}	1.7×10^{-4}
Ornithine	5.6×10^{-3}	2.3×10^{-3}	9.3×10^{-3}	6.7×10^{-4}
Glutamin	5.3×10^{-4}	8.9×10^{-5}	7.7×10^{-4}	5.4×10^{-5}
Metformin	3.6×10^{-3}	4.2×10^{-4}	8.5×10^{-4}	5.6×10^{-5}
Gliclazide	4.2×10^{-4}	3.5×10^{-5}	5.3×10^{-4}	5.3×10^{-5}

3.6. Effect of temperature on the sensors performance

The effect of temperature of the test solution on sensor performance was studied. This was carried out by plotting the calibration graphs (sensor potential vs. p^{PLZ}) at different test solution temperatures (25, 30, 40, 50, 60 and 70˚C) for all sensors. Table 4 summarized the slopes, usable concentration ranges and the standard sensor potential (E°) at each temperature.

To determine the isothermal coefficients (dE°/dt) of the sensors, the standard sensor potentials (E°) against the normal hydrogen electrode at the different temperatures were measured. This can be carried out by plotting the intercepts at $p^{PLZ} = 0$ (after subtracting the values of the standard sensor potential of Ag/ AgCl electrode at these temperatures) vs, (*t-25*) where *t* is the temperature of test solution in $^\circ$C. The obtained straight line was, according to the following equation.[30]

$$E^\circ = E^\circ(25) + (dE^\circ/dt)\,(t-25)$$

The isothermal coefficients were represented from the slopes of the straight lines obtained. They were amounted to 0.00163, 0.00037, 0.00089 and 0.00985 $V^\circ C^{-1}$ for sensors I, II, III

and IV, respectively. These low values were revealed that the PLZ-TPB sensors have high thermal stability within the studied temperature range.

Table 4: Performance characteristics of PLZ-TPB sensors at different temperatures.

Type of sensors	Temperature °C	Slope/mV decade^{-1}	Usable concentration range	E°/mV[a]
PLZ-TPB Coated wire (sensor I)	25	53.8	1.0×10^{-6}-1.0×10^{-3}	150
	30	56.2	1.0×10^{-5}-1.0×10-2	158
	40	59.0	1.0×10^{-5}-1.0×10^{-2}	170
	50	61.4	1.0×10^{-5}-1.0×10^{-2}	190
	60	63.8	1.0×10^{-5}-1.0×10^{-2}	212
	70	65.6	1.0×10^{-4}-1.0×10^{-2}	218
PLZ-TPB modified β-CD (sensor II)	25	55.5	1.0×10^{-6}-1.0×10^{-2}	320
	30	58.7	1.0×10^{-5}-1.0×10^{-3}	326
	40	60.2	1.0×10^{-5}-1.0×10^{-2}	340
	50	62.5	1.0×10^{-5}-1.0×10^{-2}	354
	60	64.8	1.0×10^{-5}-1.0×10^{-2}	366
	70	66.2	1.0×10^{-4}-1.0×10^{-2}	378
PLZ-TPB carbon paste (sensor III)	25	57.1	1.0×10^{-7}-1.0×10^{-2}	430
	30	59.4	1.0×10^{-6}-1.0×10^{-2}	437
	40	60.6	5.0×10^{-6}-1.0×10^{-2}	451
	50	63.3	5.0×10^{-6}-1.0×10^{-2}	467
	60	65.2	1.0×10^{-5}-1.0×10^{-2}	482
	70	67.4	1.0×10^{-5}-1.0×10^{-2}	497
PLZ-TPB modified CNTs (sensor IV)	25	57.7	1.0×10^{-8}-1.0×10^{-2}	560
	30	59.8	5.0×10^{-7}-1.0×10^{-2}	563
	40	61.2	5.0×10^{-6}-1.0×10^{-2}	569
	50	63.5	5.0×10^{-6}-1.0×10^{-2}	577
	60	64.8	5.0×10^{-6}-1.0×10^{-2}	584
	70	66.3	1.0×10^{-5}-1.0×10^{-2}	589

[a]***E°: Standard sensor potential against normal hydrogen electrode (NHE)***

3.7. Analytical applications

3.7.1. Quantification of pioglitazone hydrochloride

PLZ was quantified directly in bulk form using PLZ-TPB sensors. The mean % recoveries were recorded. The obtained results were 98.9±0.7, 99.1±0.7, 99.4±0.5 and 99.7±0.3 for sensors I, II, III and IV, respectively (Table 5). Furthermore, the proposed method was applied for the determination of PLZ in its pharmaceutical preparations; the results were 98.9±0.3, 99.3±0.6, 99.1±1.1 and 99.7±0.3 for the above mentioned sensors (Table 6).

Table 5: Statistical treatment of data obtained by determination of PLZ in bulk drug using PLZ-TPB sensors.

Sample	PLZ-TPB coated wire (sensor I)			PLZ-TPB coated wire Modified β-CD (sensor II)			PLZ-TPB carbon paste (sensor III)			PLZ-TPB modified CNTs (sensor IV)		
	Taken -log conc. (mol L^{-1})	Found	% Recovery	Taken -log conc. (mol L^{-1})	Found	% Recovery	Taken -log conc. (mol L^{-1})	Found	% Recovery	Taken -log conc. (mol L^{-1})	Found	% Recovery
Pure drug	6.0	5.86	97.6	6.0	5.99	99.8	7.0	6.98	99.7	8.0	7.98	99.7
	5.3	5.26	99.2	5.0	4.96	99.2	6.0	5.99	99.8	7.0	6.99	99.8
	5.0	4.99	99.6	4.0	3.98	99.5	5.0	4.96	99.2	6.0	6.00	100.0
	4.3	4.23	98.4	3.3	3.24	98.1	4.0	3.94	98.5	5.0	4.96	99.2
	4.0	3.96	99.0	3.0	2.95	98.3	3.0	3.00	100.0	4.0	3.97	99.3
	3.0	2.98	99.3	2.0	1.99	99.5	2.0	1.98	99.0	3.0	2.99	99.7
										2.0	2.00	100.0
%Mean	98.9±0.7			99.1±0.7			99.4±0.5			99.7±0.3		
SD	0.7			0.7			0.5			0.3		
n	6			6			6			7		
Variance	0.49			0.49			0.25			0.09		
%SE	0.29			0.29			0.20			0.11		
%RSD	0.71			0.70			0.50			0.30		

Table 6: Statistical treatment of data obtained by determination of PLZ in Glitazone® 15 mg /tablet using PLZ-TPB sensors.

Sample	PLZ-TPB coated wire (sensor I)			PLZ-TPB coated wire Modified β-CD (sensor II)			PLZ-TPB carbon paste (sensor III)			PLZ-TPB modified CNTs (sensor IV)		
	Taken -log conc. (mol L^{-1})	Found	% Recovery	Taken -log conc. (mol L^{-1})	Found	% Recovery	Taken -log conc. (mol L^{-1})	Found	% Recovery	Taken -log conc. (mol L^{-1})	Found	% Recovery
Glitazone® tablets	6.0	5.95	99.2	6.0	5.98	99.6	7.0	6.99	99.9	8.0	7.99	99.8
	5.3	5.24	98.9	5.0	4.93	98.6	6.0	6.00	100.0	7.0	6.98	99.7
	5.0	4.97	99.4	4.0	3.94	98.5	5.0	4.98	99.6	6.0	5.99	99.8
	4.3	4.25	98.8	3.3	3.30	100.0	4.0	3.99	99.8	5.0	5.00	100.0
	4.0	3.94	98.5	3.0	2.99	99.7	3.0	2.94	98.0	4.0	4.00	100.0
	3.0	2.96	98.7	2.0	1.99	99.5	2.0	1.95	97.5	3.0	2.99	99.7
										2.0	1.98	99.0
%Mean	98.9±0.3			99.3±0.6			99.1±1.1			99.7±0.3		
SD	0.3			0.6			1.1			0.3		
n	6			6			6			7		
Variance	0.09			0.36			1.21			0.09		
%SE	0.12			0.24			0.44			0.11		
%RSD	0.30			0.60			1.10			0.30		

The proposed method was successfully applied for the determination of PLZ in biological fluids. The results obtained for determination of PLZ in urine were 98.6±0.8, 99.3±0.4, 99.3±0.6 and 99.6±0.5 while in human serum the recorded results were 98.7±0.5, 99.2±0.6, 99.3±0.8 and 99.8±0.3 for sensors I, II, III and IV, respectively (Tables 7 and 8).

Table 7: Statistical treatment of data obtained by the determination of PLZ in urine samples using PLZ-TPB sensors.

Sample	PLZ-TPB coated wire (sensor I)			PLZ-TPB coated wire Modified β-CD (sensor II)			PLZ-TPB carbon paste (sensor III)			PLZ-TPB modified CNTs (sensor IV)		
	Taken -log conc. (mol L^{-1})	Found	% Recovery	Taken -log conc. (mol L^{-1})	Found	% Recovery	Taken -log conc. (mol L^{-1})	Found	% Recovery	Taken -log conc. (mol L^{-1})	Found	% Recovery
Glitazone® tablets	5.0	4.99	99.8	6.0	5.95	99.2	7.0	6.98	99.7	8.0	7.98	99.8
	4.3	4.25	98.8	5.0	4.96	99.2	6.0	5.99	99.8	7.0	6.96	99.4
	4.0	3.95	98.7	4.0	3.95	98.8	5.0	4.96	99.2	6.0	6.00	100.0
	3.3	3.22	97.6	3.3	3.30	100.0	4.0	3.97	99.3	5.0	5.00	100.0
	3.0	2.94	98.0	3.0	2.98	99.3	3.0	2.95	98.3	4.0	3.95	98.8
										3.0	2.98	99.3
%Mean	98.6±0.8			99.3±0.4			99.3±0.6			99.6±0.5		
SD	0.8			0.4			0.6			0.5		
n	5			5			5			6		
Variance	0.64			0.16			0.36			0.25		
%SE	0.36			0.18			0.27			0.20		
%RSD	0.80			0.40			0.60			0.50		

Table 8: Statistical treatment of data obtained by determination of PLZ in serum samples using PLZ-TPB sensors.

Sample	PLZ-TPB coated wire (sensor I)			PLZ-TPB coated wire Modified β-CD (sensor II)			PLZ-TPB carbon paste (sensor III)			PLZ-TPB modified CNTs (sensor IV)		
	Taken -Log conch. (mol L^{-1})	Found	% Recovery	Taken -log conc. (mol L^{-1})	Found	% Recovery	Taken -log conc. (mol L^{-1})	Found	% Recovery	Taken -log conc. (mol L^{-1})	Found	% Recovery
Glitazone® tablets	5.0	4.96	99.2	6.0	5.97	99.5	7.0	7.00	100.0	8.0	7.98	99.8
	4.3	4.25	98.8	5.0	4.98	99.6	6.0	5.96	99.3	7.0	7.00	100.0
	4.0	3.96	99.0	4.0	3.94	98.5	5.0	4.98	99.6	6.0	6.00	100.0
	3.3	3.24	98.1	3.3	3.30	100.0	4.0	3.99	99.8	5.0	4.96	99.2
	3.0	2.95	98.3	3.0	2.96	98.7	3.0	2.94	98.0	4.0	4.00	100.0
										3.0	2.99	99.7
%Mean	98.7±0.5			99.2±0.6			99.3±0.8			99.8±0.3		
SD	0.5			0.6			0.8			0.3		
n	5			5			5			6		
Variance	0.25			0.36			0.64			0.09		
%SE	0.22			0.27			0.36			0.11		
%RSD	0.50			0.60			0.80			0.30		

The evaluation of the proposed method was carried out by applying statistical analysis for the obtained results using student's *t*- and *F*- tests at 95.0% confidence level. [31] Table 9 showed that the obtained results were in good agreement with those obtained from a reported method (a spectrophotometric method which is based on the reaction of pioglitazone hydrochloride with tetrazolium blue reagent in presence of sodium hydroxide at 65 ºC to form a pink-violet product with maximum absorbance at 518 nm). [18]

Table 9: Statistical treatment of data obtained for the determination of PLZ in Glitazide® (15 mg/ tablet) by proposed and reported method [18] using standard addition method.

Type of sensor	Taken mol L^{-1}	Mean%±SD	n	Variance	SE	%RSD	*t*-test	*F*-test
PLZ-TPB Coated wire (sensor I)	1.0×10^{-6}-1.0×10^{-3}	98.9±0.6	6	0.36	0.24	0.6	1.376 (2.228)*	2.25 (5.05)*
PLZ-TPB modified β-CD (sensor II)	1.0×10^{-6}-1.0×10^{-2}	99.3±0.5	6	0.25	0.20	0.5	1.189 (2.228)*	2.78 (5.05)*
PLZ-TPB carbon paste (sensor III)	1.0×10^{-7}-1.0×10^{-2}	99.6±0.7	6	0.49	0.28	0.7	0.431 (2.228)*	1.65 (5.05)*
PLZ-TPB modified CNTs (sensor IV)	1.0×10^{-8}-1.0×10^{-2}	99.7±0.8	7	0.64	0.30	0.8	0.209 (2.201)*	1.27 (4.39)*
Reported method [18]	1.0×10^{-6}-1.0×10^{-2}	99.8±0.9	6	0.81	0.37	0.9		

*** Figures in parentheses are the tabulated values of t- and F- testes [31]**

Content uniformity assay of the tablets

The content uniformity assay of PLZ in its tablets (Glitazide® 15 mg/tablet) was determined using the fabricated PLZ- TPB sensors. To study the content uniformity assay of PLZ tables, ten individual tablets were placed in separate 100-mL beakers and dissolved in 100 mL distilled water. The fabricated sensors were used directly to measure the investigated drug. The mean potential was used to evaluate the content uniformity from the calibration graph. The obtained results as % recoveries ± standard deviations were 98.2±0.4, 99.2±0.2, 99.5±0.4 and 99.8±0.2 for sensors I, II, III and IV, respectively.

Determination of pioglitazone hydrochloride in presence of its degradation products

The fabricated PLZ-TPB sensors were used for determination of PLZ in the presence of its degradation products. The potential readings for the fabricated sensors using 1.0×10^{-3} mol L^{-1} in the presence of (20, 40, 60, 80 and 100 %) degradation products were compared with those obtained by 1.0×10^{-3} mol L^{-1} pure PLZ solution. It was found that the mean % recoveries were 98.1±0.6, 99.0±0.4, 99.4±0.8 and 99.8±0.4 for sensors I, II, III and IV, respectively, indicating good selectivity and high sensitivity of the fabricated sensors for determination of PLZ even in the presence of its degradation products.

3.8. Method validation

The validation parameters were studied according to ICH guidelines[27] to assess the performance of the proposed method. Linearity, lower limit of detection, accuracy, precision, robustness and ruggedness were investigated.

3.8.1. Linearity and lower limit of detection

The linearity of the proposed method was tested using PLZ test solutions of concentrations ranging from 1.0×10^{-8} -1.0×10^{-1} mol L^{-1}. The drug test solutions were subjected to PLZ-TPB sensor detection systems. The linearity was determined by plotting the sensor potentials against –log concentration of PLZ. The results obtained clarified that the constructed sensors displayed Nernstian response over linear concentration ranges of 1.0×10^{-6}-1.0×10^{-3}, 1.0×10^{-6}-1.0×10^{-2} mol L^{-1}, 1.0×10^{-7}-1.0×10^{-2} and 1.0×10^{-8}-1.0×10^{-2} mol L^{-1} for sensors I, II, III and IV, respectively. From the obtained results, the use of 2-hydroxypropyl-β CD increases the sensitivity of the membrane towards PLZ cation and the linear concentration range was increased for sensor II more than sensor I. While, in the case of carbon paste sensors the use of the porus large surface area of modified carbon nanotubes sensor and due to its best sensor electrolyte interface, carbon nanotubes improved the performance of the sensor for the detection of small concentrations of PLZ. The detection limit was calculated according to IUPAC recommendation which stated that the detection limit is the concentration at which the measured potential differs from that predicted by the linear regression by more than 18 mV. The detection limits were found to be about 5.0×10^{-7}, 4.7×10^{-7}, 5.0×10^{-8} and 4.8×10^{-9} mol L^{-1} for sensors I, II, III and IV, respectively.

3.8.2. Accuracy and precision

The accuracy of the proposed method was tested by determining the investigated drug in the presence of its coformulated substance lactose using the standard addition method. The obtained results were calculated in terms of % recovery values. The calculated % recoveries were 98.7±0.9, 99.2±0.4, 99.5±0.5 and 99.6±0.2 for sensors I, II, III and IV, respectively.

To investigate the precision of the proposed method, intra-day and inter-day terms were used. The studies were performed by repeating the determination to nine replicates. The calculated %RSD values were 0.42, 0.35, 0.24 and 0.17 % for determination of PLZ in (Glitazone® 15 mg/tablet) using PLZ- TPB sensors I, II, III and IV, respectively. The above %RSD values are less than 2% indicating good precision.

3.8.3. Robustness and ruggedness

To evaluate the robustness of the proposed method phosphate buffer of pH 8.0 was used to introduce small changes in pH. The percentage recoveries were calculated for the investigated drug solutions at pH 8.0±1. The obtained results were 98.9±0.8, 99.5±0.5, 99.7±0.2 and 99.9±0.1 for sensors I, II, III and IV, respectively. These results were closely in agreement with those obtained from standard drug solutions. The ruggedness of the proposed method was tested using another pH-meter (Jenway 3510). The recorded results as % recoveries were 98.6±0.5, 99.2±0.1, 99.4±0.7 and 99.8±0.4 for the previously mentioned sensors, respectively.

4. CONCLUSION

This study described a simple, sensitive and accurate electrochemical method for the determination of PLZ. The electrochemical method was based on the fabrication of four different sensors. Sensors I and II were PLZ-TPB coated wire membrane and sensor II was modified using 2-hydroxypropyl β-cyclodextrin. Sensors III and IV were carbon paste type and fabricated using the same ion pair while, sensor IV was modified using carbon nanotubes. The described sensors are sufficiently simple and selective for the quantitative determination of PLZ in bulk form, its pharmaceutical formulations and biological fluids. The use of 2-hydroxy propyl β-cyclodextrin as ionophore increased the membrane sensitivity and selectivity of sensor II more than sensor I. Furthermore, the use of modified carbon nanotubes carbon paste in sensor IV provided higher sensitivity, lower limit of detection, faster dynamic response time and wide concentration range than sensor III and other fabricated types. The statistical data analysis proved successful agreement with those obtained from other reported method.

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