Research Article

Assurance of Nano-Molar Amounts of a Tricyclic Antidepressant; Clomipramine Hydrochloride in Bulk, Pharmaceutics and Biological Fluids Utilizing Solid Contact Sensors

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ABSTRACT

Clomipramine hydrochloride (CLPH.Cl) as a tricyclic antidepressant was analyzed utilizing newly constructed sensitive coated wire sensors. These sensors are based on the use of clomipramine-tetraphenylborate (CLPH-TPB) as the electroactive matter. A mixture of 2.0% CLPH-TPB, 49.0% polyvinyl chloride (PVC) and 49.0% tricresyl phosphate (TCP) was used. Distinctive wires and graphite pencils were checked for the closest Nernastian behavior. The sensors showed a slope of 58.71±0.27 mV/decade over a linear concentration range of 1.0×10^{-3}-10.0 mmol/L with detection limit of 0.49 µmol/L and correlation coefficient 0.9989. Different additives are incorporated in the prepared mixture (Nano carbon, potassium tetraphenylborate or sodium tetraphenylborate) to enhance the sensor characteristics. The sensors exhibit stable potential within the pH range 2.0-7.2 and show steady state potentials within 5-10 s. Additionally, the proposed sensors were utilized for the assurance of CLPH.Cl in pure form, pharmaceutics (Anafronil®tablets) and biological fluids (spiked human serum and urine) reaching a limit of detection of 30.0 nmol/L on the basis of standard addition method. The obtained recovery values lay in the range 98.28-103.66%. The results were statistically compared with the British pharmacopeial method, demonstrating no critical distinction with respect to its accuracy and precision.

Keywords: Clomipramine hydrochloride, Polyvinyl chloride, solid contact sensor, Nano carbon.

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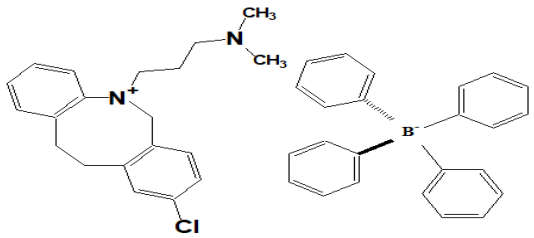
INTRODUCTION

Clomipramine hydrochloride (CLPH.Cl) belongs to tricyclic antidepressants which are broadly used to treat real depressive disorder and significant anxiety disorder[1] by inhibiting the reuptake of serotonin and hindering its transporters[2]. Its name is 3-(3-chloro-10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-N,N-dimethylpropan-1-amine hydrochloride. Its molar mass is 351.3 g mol^{-1}. Different systematic techniques have been reported for the quantification of CLPH.Cl including spectral analyses, [3-8] chromatography. [9-16] The majority of these strategies are tedious techniques and in light of utilizing of refined instrument. Consequently, suggesting an electrochemical strategy utilizing an ion-selective sensor is a decent applying elective technique for the medication investigation. Ion-selective sensors are assuming an imperative investigation due to its simplicity, rapidity and accuracy over some other analytical technique. [17-21] Some previously published ion selective sensors were constructed for the quantification of CLPH.Cl in the pure and pharmaceutical formulations utilizing membrane and chemically modified carbon paste sensors [23-26] and they were summarized in our previously published work. [23]

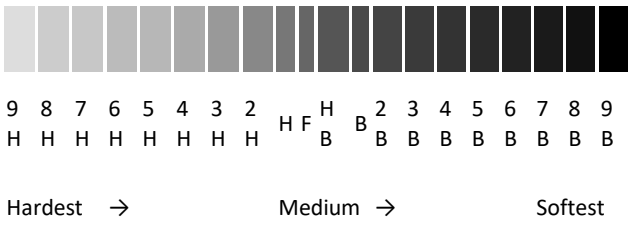
The present work expects to create coated solid contact sensors for the assurance of CLPH.Cl in the pure, pharmaceutical and biological samples where there is no previously published sensor for its analysis from this type. Besides, it studies the performance characteristics and the applicability in the analysis of pure solutions, pharmaceutics, spiked human serum and urine samples utilizing potentiometric titration and standard addition methods. The proposed CLPH-sensors were produced on the view of fuse of CLPH-TPB, scheme 1, as the electroactive matter in a plasticized PVC to get ready layer and covered wire sensors. They were characterized and upgraded according to IUPAC suggestions. [22]



Scheme 1: Chemical structure of clomipramine hydrochloride -tetra phenyl borate ion pair

Graphite is considered as the astounding level of coal, which has been used in the development of sensors because of its thermal and electrical conductivity. The

hardness of graphite pencils contrasts relying upon its quality and where the pencil was made. Delicate lead was labeled "B" for black and "H" for hard. "F" is a letter chosen to indicate midway between HB and H. An HB pencil is in the half way amongst hard and delicate. Scheme 2 shows the distinctive grades of graphite pencils.



Scheme 2: Diverse grades of graphite pencils in plummeting request as per their hardness.

MATERIALS AND METHODS

Reagents and Materials

Clompramine HCl and its pharmaceutics (Anafronil® tablets, 25 and 50 mg/tablet) were obtained from NOVARTIS PHARMA S.A.E. Cairo, Egypt. Dibutyl phthalate (DBP), ethylhexyladipate (EHA), dioctyl phthalate (DOP), dioctylsebacate (DOS) o-nitrophenyl phenyl ether (oNPPE), tricresyl phosphate (TCP), o-nitrophenyloctyl ether (oNPOE) and sodium tetraphenylborate (NaTPB), were gotten from Sigma-Aldrich, USA. All chemicals and reagents were of analytical grade. Doubly distilled water was utilized through all the work.[23]

Solutions

The working standard solutions were set up by appropriate dilution of the stock 10.0 mmol/L CLPH.Cl solution. The later was set up by dissolving, 35.13 mg in 100 mL doubly distilled water. A 10.0 mmol/L NaTPB standard solution was prepared by dissolving 34.22 mg into 100 mL doubly distilled water. Solutions of sodium hydroxide and hydrochloric acid of concentrations within the range 0.1-1.0 mol/L were utilized for altering the pH of the medium. The stock solutions and the diluted ones were kept in dark brown bottles in the fridge.

To explore the selectivity of the proposed sensors, 0.1 mol/L lactose, sucrose, DL-asparagine, L-threonine, D-alanine, DL-histidine, L-cysteine, and DL-serine (gotten from Aldrich Chemical Company), chloride solutions of Na^+, Ca^{2+}, Cu^{2+}, K^+, NH_4^+ and Mg^{2+} and nitrate solution of Fe^{3+}, (gotten from Adwic Chemical Company, Abu-Zabal, Egypt), were prepared. [23]

Preparation of Clomipramine-Tetraphenylborate (CLPH-TPB)

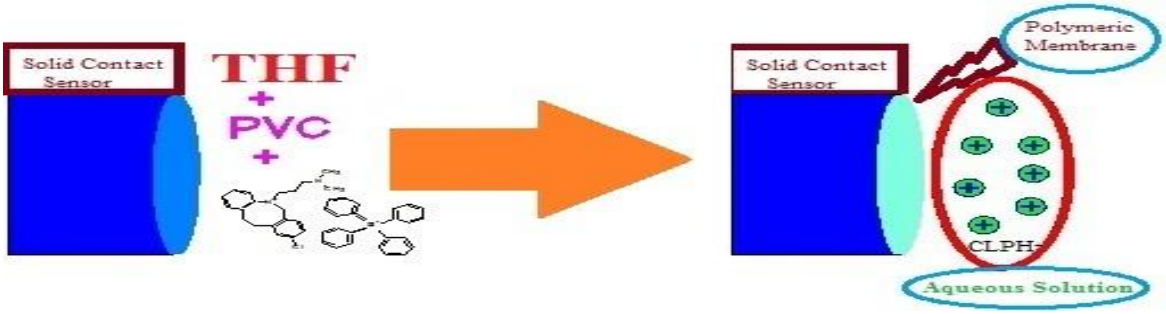
It was prepared as described in our previously work[23] by blinding 50 mL 10.0 mmol/L CLPH.Cl with 50 mL 10.0 mmol/L NaTPB, under stirring.

Preparation of Sensor Coating Mixture

The blend was prepared by dissolving diverse amounts of CLPH-TPB covering the range of 1.0-5.0%, distinct amounts of PVC and different types of plasticizers in 5 mL tetrahydrofuran (THF). The aggregate weight of constituent in each cluster is settled at 200 mg.

Preparation of Coated Wire Sensors

Unadulterated silver (2 mm diameter and 6 cm length), copper (3 mm diameter and 6 cm length), glassy carbon (GC) (5 mm diameter and 6 cm length) and pencil graphite rods (2 mm diameter and 6 cm length) of diverse grades were tightly insulated using poly ethylene tubes leaving 2 cm at one end for coating and 1 cm at the other end for connection. The rod surface of each type was coated with the active membrane by ducking the uncovered end into the coating solution and allowing the film to dry in air for about 5 min. The process was done until a plastic film of around 1.0 mm thickness was formed (around 10 times). The prepared sensors were preconditioned by soaking in 1.0 mmol/L solution of CLPH.Cl for various time interims.



Scheme 2: Schematic representation for the preparation of solid contact sensors

Instrumentation

The potentiometric and pH estimations were done with a Jenway 3010 digital pH/mV meter. A saturated calomel electrode (SCE) was used as the external reference. The electrochemical arrangement of the sensors would be represented as sensor|test solution|SCE. The temperature was adjusted utilizing Thermostat, Model C-100 (Cambridge, England).

Effect of pH

The effect of pH on the response of the prepared sensors was tested using 0.1, 0.5 and 1.0 mmol/L CLPH.Cl. The pH of the solution was covered the range 1.0-12.0 by adding small amounts of 1.0 mol/L HCl or NaOH solutions. The emf readings were drawn against the pH for the diverse drug concentrations.

Effect of Temperature

Calibration graphs were constructed at different test solution temperatures (t) (25-55 ºC) to look for the thermal coefficient of the suggested sensors. The standard sensor potential values (E°_{sen}) at each temperature were calculated in this temperature range. The values of Eº were plotted versus (t-25). The slope of those straight lines presents the thermal coefficient (dEº/dt) which was computed for each sensor using equation (1):

$$E^{\circ}_{cell}=E^{\circ}_{25^{\circ}C} + (dE^{\circ}/dt)\ (t-25) \quad (1)$$

Plot of E°_{cell} versus (t-25) produced a straight line whose slope is taken as the thermal coefficient of the cell. The values of the standard potentials of the sensors (E°_{sen}) were calculated after subtraction of the potential of the SCE at different temperatures. [27]

Response to other Ions

The selectivity coefficient values $\left(K^{pot}_{CLPH^{+},j^{z+}}\right)$ were resolved by IUPAC suggestions utilizing the matched potential strategy.[26] It is known as the ratio of essential and interferent which obtain the similar potential change under comparable conditions. $K^{pot}_{CLPH^{+},j^{z+}}$ was determined by applying equation (2):[28]

$$K^{pot}_{CLPH^{+},j^{z+}} = (a_{CLPH})/(a_j) \quad (2)$$

Where a_{CLPH} and a_j: the activity of the drug and the added interferent, respectively.

Analytical Applications

CLPH.Cl was analyzed in the pure form, pharmaceutical preparation (Anafronil® tablets) and spiked human serum and urine samples applying the standard addition and potentiometric titration methods. The standard addition method was carried out by adding small volumes of pure CLPH.Cl solution to an aliquot of samples of diverse concentrations covering the range from 0.1 to1.0×10^{4} µmol/L. The potential reading was observed for each and every increment and used to report the concentration in the tested solution utilizing equation (3).[29]

$$C_x = C_s \left(\frac{Vs}{Vx+Vs}\right) \left(10^{n(\Delta E/S)} - \frac{Vx}{Vs+Vx}\right)^{-1} \quad (3)$$

Where:

Cx, Cs,Vx, and Vs: are the concentrations to be resolved, the concentration of standard CLPH.Cl included, the starting volume and the volume included of standard CLPH.Cl, respectively.

S: is the slope of the alignment bend

ΔE: is the potential change after expansion.

Making utilization of the potentiometric titration, distinctive volumes from 10.0 mmol/L CLPH.Cl were weakened to 50 mL utilizing doubly distilled water and the subsequent arrangements were titrated against 1.0 or 10.0 mmol/L NaTPB. The emf esteems were processed and plotted against the volume of NaTPB (V). The end focuses were distinguished from the customary S-formed plots and the first derivative.

Investigation of Tablets

Ten Anafronil® tablets were precisely weighed and ground to fine powder in an agate mortar and a known weight was taken from this powder and blended with 30 mL of doubly distilled water at that point, the later was separated in 50 mL estimating flask. The remained powder was washed three times with a similar dissolvable and the volume was finished to the check. Following that, the standard addition and potentiometric titration techniques were connected.

Investigation of Serum and Urine Samples

Intended for serum investigation, about 4 mL of blood was centrifuged for 10 min at 1500 rpm, and then one mL serum was transferred to a 50 mL estimating flask. For urine investigation, one mL aliquot of it was moved into 50 mL estimating flask. Diverse volumes of 10.0 mmol/L CLPH.Cl solution were included so the last fixation was at the scope of 0.1-100.0 µmol/L. The later were mixed well and completed to volume level with doubly distilled water. The later were blended well and finished to volume level with doubly distilled water. These arrangements were dissected as depicted above utilizing the standard addition strategy.

RESULTS AND DISCUSSION

Performance Characteristics of the Sensors

To choose the best composition of the readied covering blend, CLPH-TPB (1-5%) were utilized to acquire the ideal creations and tried as a membrane sensor. The later was set up by pouring the mixture into a Petri dish (5 cm in measurement), secured with a filter paper and the dissolvable was permitted to dissipate gradually at room temperature. For every circumstance, consequent to curing little circles (7.5 mm in estimation) were punched from the thrown films and mounted in a natively constructed sensor boat. The sensor was filled with 10.0 mmol/L KCl and 1.0 mmol/L CLPH.Cl then it was preconditioned in 1.0 mmol/L CLPH.Cl solution.

The best outcome was gotten from mixing 2% CLPH-TPB, Table 1. The impact of various plasticizers on the performance qualities of the sensor, with creation of 2% CLPH-TPB, was contemplated, Table 2. The information demonstrates that the utilization of TCP as a plasticizer gave the best outcomes with the closest Nernstian slope (56.28±0.08 mV/decade) over a wide range (5.96×10^{-3}-10.0 mmol/L) with LOD and LOQ 5.38 and 10.79 µmol/L, individually. Where on account of different plasticizers, the slope of the adjustment charts is entirely different from the normal Nernstian esteem; 59.5 mV/decade. Diverse wires were then tried utilizing the achieved composition; 2.0% CLPH-TPB, 49.0% PVC and 49.0% TCP. The slopes, concentration ranges and detection limits of solid contact sensors are affected by the idea of the bed. Ag and Cu, GC and graphite pencils (GHB) are utilized for this reason.

Table 1: Performance characteristics of the CLPH$^+$ membrane sensors at 25±1.0 ºC.

Composition(w/w%)			Slope ± SE	Linear range	LOD	RSD*	r^2
I.P	Plast.	PVC	mV/decade	mmol/L	µmol/L	%	
1	49.5 DBP	49.5	50.20±0.37	9.90×10^{-3}-10.0	9.32	1.30	0.9987
2[a]	49.0 DBP	49.0	52.43±0.28	9.90×10^{-3}-10.0	6.17	0.95	0.9997
3	48.5 DBP	48.5	51.72±0.31	1.96×10^{-2}-10.0	147.00	1.06	0.9992
5	47.5 DBP	47.5	50.62±0.27	2.53×10^{-2}-10.0	1.67	0.95	0.9986
2	49.0 DOS	49.0	55.92±0.15	1.17×10^{-2}-10.0	8.88	0.48	0.9983
2[b]	49.0 TCP	49.0	56.28±0.08	5.96×10^{-3}-10.0	5.38	0.24	0.9998
2	49.0 oNPOE	49.0	51.63±0.14	1.96×10^{-2}-10.0	1.76	0.54	0.9994
2	49.0 DOP	49.0	53.45±0.35	7.93×10^{-3}-10.0	8.10	1.15	0.9990
2	49.0 EHA	49.0	52.66±0.35	9.90×10^{-2}-10.0	140.00	1.15	0.9996

[a] the best composition, [b] the selected membrane sensor, *RSD of four replicate measurements, plast.: plasticizer, , SE: standard error, r^2: correlation coefficient, I.P: CLPH-TPB

The impact of three sorts of added substances like Nano carbon (NC), potassium tetraphenylborate (KTPB) and NaTPB on the reaction qualities of solid contact sensors was considered. Consolidation of added substances genuinely influences the performance attributes of CLPH-TPB solid contact sensors particularly GHB sensor. Table 2 and Fig 1 demonstrated that, for GC based sensor, the LOD diminished fundamentally however the slope value is steady or diminished. For GHB based sensor, on including 0.5% KTPB, the slope value enhanced from 52.09±0.21 to 58.71±0.27 mV/decade, the LOD reduced to 0.49 µmol/L, and the range is 1.0×10^{-3}-10.0 mmol/L. Then again, if there should arise an occurrence of different added substances slopes, ranges and LODs stay consistent or don't change altogether. The impact is unbeneficial if there should arise an occurrence of Ag and Cu CWSs in light of the fact that the slope value diminished, the range and the LOD stay consistent or don't change fundamentally. Clearly the GHB rod with incorporation of 2.0% CLPH-TPB+0.5% KTPB+48.75% PVC+48.75% TCP (GHB/KTPB) is the best solid contact sensor utilized all through this examination.

Table 2: Effect of different additives percentage on the performance characteristics of CLPH-TPB solid contact sensors.

Additive	Slope±S.E	Linear range	LOD	RSD	r^2
%	mV/decade	mmol/L	µmol/L	%	
GC					
----	56.97±0.20	5.96×10^{-3} -10.0	2.34	0.71	0.9988
0.5 NC	54.25±0.31	3.90×10^{-3} -10.0	1.42	1.15	0.9953
1.0 NC	52.70±0.18	3.98×10^{-3} -10.0	1.08	0.69	0.9968
0.5 KTPB	56.63±0.23	7.50×10^{-4} -10.0	0.36	0.83	0.9990
1.0 KTPB	55.84±0.16	1.19×10^{-3} -10.0	0.75	0.61	0.9993
0.5 NaTPB	56.24±0.06	7.99×10^{-4} -10.0	0.61	0.24	0.9979
1.0 NaTPB	52.74±0.14	3.99×10^{-4} -10.0	0.27	0.57	0.9987
GHB					
----	52.38±0.22	5.96×10^{-3}-10.0	1.85	0.87	0.9987
0.5 NC	52.59±0.26	3.98×10^{-3}-10.0	1.27	1.01	0.9936
1.0 NC	52.09±0.21	1.99×10^{-3}-10.0	0.97	0.82	0.9959
*0.5 KTPB	58.71±0.27	1.00×10^{-3}-10.0	0.49	0.92	0.9989
1.0 KTPB	53.66±0.22	1.99×10^{-3}-10.0	0.84	0.85	0.9971
0.5 NaTPB	52.84±0.23	1.19×10^{-3}-10.0	0.94	0.86	0.9956
1.0 NaTPB	52.67±0.20	3.99×10^{-4}-1.0	0.25	0.77	0.9976
Ag					
----	52.48±0.28	3.98×10^{-3}-10.0	2.55	1.07	0.9982
0.5 NC	48.56±0.44	5.96×10^{-3}-10.0	3.17	1.83	0.9947

1.0 NC	44.00±.0.31	1.19×10^{-3}-10.0	0.84	1.42	0.9980
0.5 KTPB	40.84±0.49	3.98×10^{-3}-10.0	1.15	2.40	0.9959
1.0 KTPB	48.66±0.37	5.96×10^{-3}-10.0	3.98	1.52	0.9972
0.5 NaTPB	46.62±0.13	1.19×10^{-3}-10.0	0.94	0.60	0.9925
1.0 NaTPB	44.87±0.24	3.98×10^{-3}-10.0	2.19	1.08	0.9959
Cu					
----	52.72±0.27	7.93×10^{-3}- 10.0	4.03	1.05	0.9966
0.5 NC	49.61±0.18	1.99×10^{-3}-10.0	1.00	0.75	0.9979
1.0 NC	51.60±0.32	3.98×10^{-3}-10.0	2.47	1.28	0.9959
0.5 KTPB	52.61±0.34	3.98×10^{-3}-10.0	3.01	1.31	0.9961
1.0 KTPB	48.28±0.43	3.98×10^{-3}-10.0	1.88	1.78	0.9972
0.5 NaTPB	51.96±0.88	5.96×10^{-3}-10.0	3.85	1.09	0.9965
1.0 NaTPB	50.90±0.19	3.98×10^{-3}-10.0	1.87	0.75	0.9961

SE: standard error, *RSD% of four replicate measurements, r^2: correlation coefficient,

NC: nano carbon, GC: glassy carbon, GHB: graphite of type HB

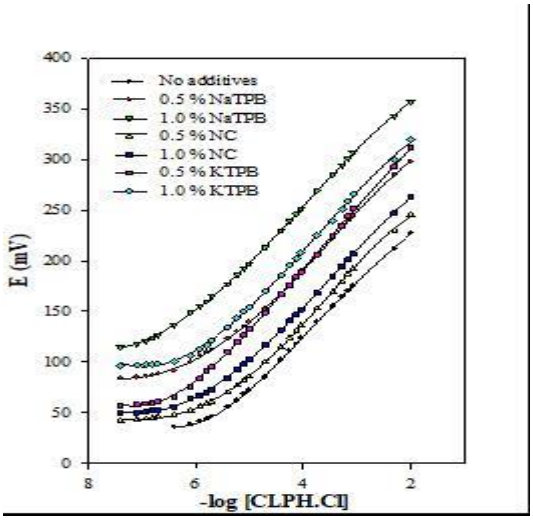


Figure 1: Effect of different percentages of additives on the CLPH-TPB GHB sensor.

The impact of splashing on the performance of the solid contact sensors was additionally examined by immersing every sensor in 1.0 mmol/L CLPH.Cl for variable time interims. The outcomes demonstrated that the life expectancies (t) of them, all in all, are equivalent or not exactly those of the comparative membrane one. This might be credited to the poor mechanical bond of the PVC-based touchy layer to the conductive bed surface and inadequate electrochemical solidness. It was discovered that the life expectancies of GC, GHB, GHB/KTPB, Cu and Ag are 15 days (slope = 56.87 mV/decade), 15 days (slope = 54.45 mV/decade), 30 days (slope = 50.54 mV/decade), 4 days (slope = 51.41 mV/decade) and 1 h (slope = 51.64 mV/decade), individually. GHB/KTPB was observed to be the best solid contact sensor which has somewhat long life time. After this period there is a sudden decline in the slope and the detection limit increases which might be ascribed to the leaching of plasticizer and CLPH-TPB from the polymeric layer into the solution[30].

Effect of pH

The impact of pH on the sensor reaction was checked utilizing three distinct concentrations of CLPH.Cl (0.1, 0.5 and 1.0 mmol/L) by estimating the variety in emf esteems with change in pH by adding little volumes of hydrochloric acid and sodium hydroxide (each 0.1-1.0 mol/L). The outcomes demonstrate that, the sensors demonstrate no potential change with respect to the pH change in the scope of 2.0-7.5, Fig. 2. At higher pH esteems the potential reductions bit by bit and this can presumably be credited to the formation of the free base in the solution prompting the decline in its concentration[31].

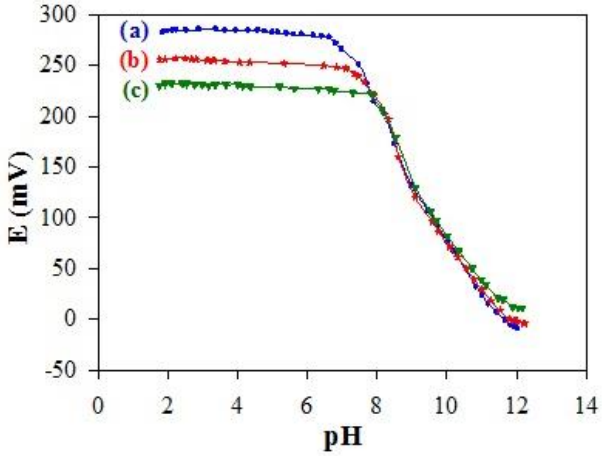


Figure 2: Effect of pH on the potential response of GHB/KTPB sensors, in (a) 1.0, (b) 0.5 and (c) 0.1 mmol/L CLPH.Cl solutions.

The response time is a critical parameter for any sensor[22], in which the time required to accomplish an unfaltering state potential (within±1 mV) was estimated after progressive submersion of the sensor in a progression of CLPH.Cl solution each having a 10-crease increment in focus from 1.0×10^{-2} to 10.0 mmol/L. The sensor demonstrated stady state possibilities within 5-10 s.

Effect of Temperature

The test solution impacts the capacity of the sensor as showed by the Nernst condition. To examine the thermal stability of sensors, calibration diagrams were developed at various test arrangement temperatures (t) covering the scope of 25-55 ºC. The outcomes demonstrate that the slopes of the alignment diagrams still in the Nernstian run over the contemplated temperature run.

To decide the temperature coefficients of the sensors, the estimations of Eº were plotted versus (t-25). The inclines of the straight lines acquired speak to the temperature coefficients of the cells and the sensor are - 0.34, 0.73 mV/ºC, respectively. The little esteems uncover a thermal stability of the cell emf and the sensor potential inside the explored temperature go.

Response to other Ions

Response to some amino acids, essential inorganic cations and sugars which have been found in the pharmaceutical arrangements as excipients or added substances was explored. Selectivity coefficients were dictated by matched potential technique[28] utilizing film, GC and GHB/KTPB sensors. The outcomes spoke in Fig. 3 demonstrate that the got selectivity coefficients are little and this shows a high selectivity of the readied sensors toward $CLPH^+$. Inorganic cations don't meddle in light due to distinctions in ionic size, versatility and penetrability contrasted and $CLPH^+$. The high selectivity over amino acids can be ascribed to the distinctions in extremity and lipophilic nature of their particles with respect to $CLPH^+$.

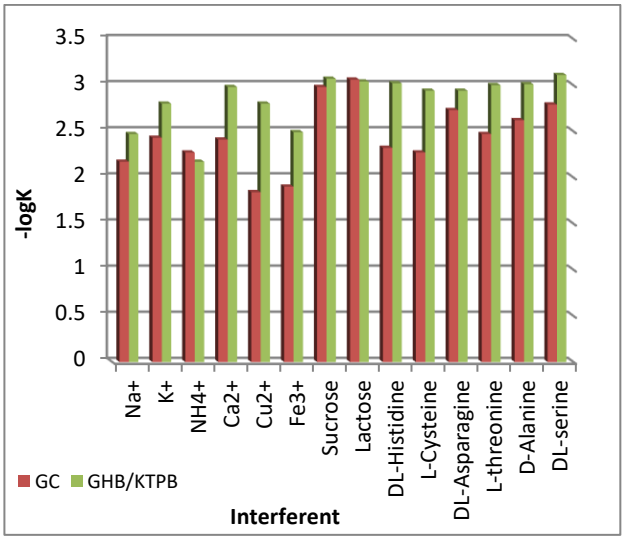


Figure 3: Selectivity coefficient ($-\log K^{pot}_{CLPH^+,j^{z+}}$) of GC and GHB/KTPB sensors

Effect of Different Graphite Grades on the Graphite Coated Wire Sensor Response Characteristics

To study the effect of graphite grade on the response characteristics of graphite coated wire sensors, different pencil rods with graphite grades HB, 2B, 4B, H and 4H were used as beds for construction of coated wire sensors with the composition of 2.0% CLPH-TPB+0.5% KTPB+48.75% PVC+48.75% TCP.

To consider the impact of graphite grade on the response attributes of graphite sensors, diverse pencil poles with graphite levels HB, 2B, 4B, H and 4H were utilized as beds for development of solid contact sensor with the creation of 2.0% CLPH-TPB+0.5% KTPB+48.75% PVC+48.75% TCP. The sensors were set up as already talked about (section 2.5) at that point, they were molded in 1.0 mmol/L CLPH.Cl for 15 min, and every sensor was inspected in the focus range 1.0×10^{-4}-10.0 mmol/L CLPH.Cl. The outcomes demonstrate that the slope values are lower than the Nernstian esteem if there should be an occurrence of G2B/KTPB (slope = 52.53±0.25 mV/decade), G4B/KTPB (slope = 51.10±0.30 mV/decade) and GH/KTPB (slope = 50.41±0.31 mV/decade) and it increases in the event of GHB/KTPB (slope = 58.08±0.20 mV/decade) and G4H/KTPB (slope = 59.39±0.23 mV/decade). From the outcomes, it was discovered that G4H/KTPB has the most Nernastian slope (59.39±0.23 mV/decade) and LOD esteems (1.20 µmol/L). In addition, GHB/KTPB has slope of 58.08±0.20 mV/decade and the low LOD esteem 0.49 µmol/L.

From the obtained results, it was presumed that as the softness of the graphite rods increases from 2B to 4B, the slope decreases and LOD values increase. As the hardness of the graphite rods increases from H to 4H, the slope and LOD values increase. The mixture between H and B by ratio 1:1 gave the best outcomes.

Analytical Applications

The Potentiometric Titration

The potentiometric titration was connected to decide CLPH.Cl in pure and pharmaceutical arrangements (Anafronil® tablets) utilizing the proposed sensors. The LOD esteem was computed by changing the volume of the taken CLPH.Cl by little additions till the sensor misfortunes its affectability, it was discovered that LOD and LOQ are 0.99 and 2.99 µmol L^{-1} utilizing GHB/KTPB sensor. For examine of the pure form, the got mean recoveries are in the range 103.24-99.50% with RSD% 1.15-0.37%, Table 3. For the examination of Anafronil® tablets (25 mg/tablet and 75 mg/tablet), the got mean recoveries are in the range 99.66-98.97% with RSD% of 0.90-0.52% uncovering that there is no interference from excipients or added substances utilized as a part of tablets, Table 3.

Table 3: Determination of CLPH.Cl in pure solutions and pharmaceutical formulations applying potentiometric titrations using GHB/KTPB solid contact sensor.

Taken, mg	Recovery%±SD	RSD*
Pure solution		
1.2	103.24±0.39	0.76
1.8	101.30±0.44	0.87
3.9	100.62±0.23	0.47
10.6	102.99±0.19	0.37
25.0	99.50±0.57	1.15
35.5	99.50±0.28	0.58
Anafronil® tablet (25 mg/tablet)		
1.5	99.66±0.45	0.90
2.5	98.97±0.34	0.70
Anafronil® tablet (75 mg/tablet)		
5.0	99.16±0.48	0.97
7.5	99.20±0.25	0.52
* RSD of four repetitive measurements		

The standard addition method

The standard addition strategy was connected to measure CLPH.Cl in pure pharmaceutical arrangements (Anafronil® tablets), spiked human serum and urine samples. The LOD and LOQ are 30.0 and 99.9 nmol/L for GHB/KTPB sensors. The acquired mean recoveries are in the range 102.59-98.86% with RSD% 1.45-0.47%. The procured results, Table 4, demonstrate that the excipients or included substances used as a tablet component, human serum and urine tests have no impediment with $CLPH^+$.

Table 4: Determination of CLPH.Cl in its pure solutions, pharmaceutical formulations and biological fluids applying the standard addition method using GHB/KTPB solid contact sensor.

Taken	Recovery%±SD	RSD*
Pure solution		
1.9 µg	98.86±0.47	0.96
2.0 µg	102.59±0.24	0.47
25.0 µg	101.83±0.31	0.63
3.0 mg	101.58±0.32	0.64
Anafronil® tablet (25 mg/tablet)		
25.0 µg	101.60±0.74	1.45
2.3 mg	100.23±0.63	1.26
Anafronil® tablet (75 mg/tablet)		
25.0 µg	99.16±0.48	0.97
2.3 mg	99.00±0.57	1.16
Spiked human serum samples		
15.0 µg	102.41±0.44	0.86
1.8 mg	102.10±0.24	0.48
Spiked human urine samples		
15.0 µg	102.25±0.54	1.05
1.8 mg	102.26±0.56	1.07
* RSD of four repetitive measurements		

Validation of the Methods

Ruggedness and Robustness of the Proposed Methods

As per the USP meaning of ruggedness, the strategy is over and over performed under various test conditions to inspect the impacts of some "non-technique related" elements, for example, research laboratories, instruments, investigators, reagents, and time, without evolving the "system related" technique parameters [32]. To contemplate the ruggedness of the techniques, a correlation between the outcomes acquired by two investigators was performed. The RSD% esteem acquired by two analysts in a similar lab is 0.66%.

The impacts of little think changes in "methodology related" technique parameters, for example, operational, environmental and crest estimation/investigation parameters were investigated by robustness [32]. The later of the proposed methods was studied while the temperature of the solution was slightly changed. The recovery percentages were great under most conditions and don't demonstrate any huge change when the basic parameters were altered.

Repeatability and Reproducibility

The repeatability and reproducibility investigations of the readied sensors have been studied. For the repeatability think about, the calibration bends were completed four times for a similar sensor. For reproducibility, the sensor of a similar structure was prepared four times and the calibration bends were developed. It was discovered that RSD% fluctuated from 0.35-0.84 and 0.55-0.91%, respectively. Four repeated assurance at various fixation levels are completed utilizing the proposed sensor to test the exactness of the technique. The RSD% esteems were observed to be under 2.0%, demonstrating sensible repeatability and reproducibility of the proposed strategy.

Statistical Treatment of the Obtained Data

The outcomes were factually contrasted and those acquired by the pharmacopeial strategy [1] utilizing combined t-and F-tests, Table 5. At 95% confidence level for four replicate estimations, the figured t-and F-values did not surpass the critical values, demonstrating that there is no significant distinction between the proposed technique and the official strategy with respect to precision and accuracy.

Table 5: Statistical treatment of the obtained data for the determination of CLPH.Cl in comparison with pharmacopoeial method.

	X±S.E	RSD%*	t-value	F-value
Pharmacopoeial method	101.59±0.72	1.42		
Potentiometric titration				
GC sensor				
Pure solution	103.50±0.28	0.55	0.59	0.15
Anafronil® tablet (25 mg/tablet)	98.92±0.41	0.82	2.91	0.32
Anafronil® tablet (75 mg/tablet)	99.52±0.31	0.64	3.21	0.19
GHB/KTPB sensor				
Pure solution	101.30±0.44	0.87	0.83	0.37
Anafronil® tablet (25 mg/tablet)	98.97±0.34	0.70	0.23	3.24
Anafronil® tablet (75 mg/tablet)	99.20±0.25	0.52	3.09	0.12
Standard addition method				
GC sensor				
Pure solution	100.65±0.53	1.06	1.60	0.55
Anafronil® tablet (25 mg/tablet)	101.31±0.43	0.85	0.88	0.36
Anafronil® tablet (75 mg/tablet)	99.89±0.89	1.78	2.28	1.53
Spiked serum samples	103.56±0.51	1.00	2.91	0.51
Spiked urine samples	101.62±0.39	0.77	0.36	0.29
GHB/KTPB sensor				
Pure solution	101.76±0.36	0.72	0.27	0.25
Anafronil® tablet (25 mg/tablet)	100.23±0.63	1.26	1.34	0.77
Anafronil® tablet (75 mg/tablet)	101.13±0.32	0.65	1.34	0.20
Spiked serum samples	102.10±0.24	0.48	0.16	0.11
Spiked urine samples	102.25±0.54	1.07	0.34	0.56

X±S.E: Recovery±standard error.
t-tabulated is 4.03 at 99.0% and 5 degree of freedom.
F-tabulated is 9.82 at 95.0% confidence limit.

CONCLUSION

The present work includes the arrangement of clomipramine hydrochloride solid contact sensors in light of the usage of CLPH-TPB as an electroactive issue. The impact of various graphite grades demonstrated that pencil of HB review gave the best outcomes; has slope of 58.08±0.20 mV/decade and the low LOD value 0.49 µmol/L. These sensors were portrayed and improved by IUPAC recommendations. The selectivity thinks about uncovered that the readied sensors have high selectivity toward $CLPH^+$ over an extensive variety of different cations and essential molecules. These sensors were effectively connected for the assurance of CLPH.Cl in pure, Anafronil® tablets and spiked human serum and urine samples in the range 102.59-98.86% with RSD% <1.5%. The got comes about are in great concurrence with those got from the British pharmacopeial one. The outcomes demonstrate that the recommended sensors have a wide linearity, Nernstian slope, low LOD, LOQ esteems and long-life time. They are likewise repeatable and reproducible sensors with RSD under 2.0%.

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