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Research Article

CHEMICALLY MODIFIED CARBON PASTE SENSORS FOR DETERMINATION OF CLOMIPRAMINE HYDROCHLORIDE IN PHARMACEUTICAL FORMULATIONS

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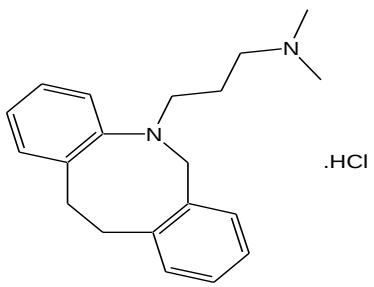
ABSTRACT

Chemically modified carbon paste sensors (CMCPSs) for determination of clomipramine hydrochloride (CLP.HCl) were prepared based on the use of ion-exchange compounds of clomipramine-tetraphenylborate(CLP-TPB) and clomipramine-silicotungestate(CLP-ST) as the electroactive materials.The potential response measurements showed that the best performance was exhibited by the sensors with composition 2% CLP-TPB, 49% o-NPPE and 49% graphite powder and 7% CLP-ST, 46.5% o-NPOE and 46.5 graphite powder.The sensors were found to be sensitive, precise and functional in the concentration ranges of 3.74×10^{-6}-1.00×10^{-2} and 3.98×10^{-6}-1.00×10^{-2}mol L^{-1}at 25±1 ºC over the pH range 2.10-7.57 and 2.00-7.34 with slopes of 60.53±0.14 and 59.22±0.12 mV $decade^{-1}$for CLP-TPB and CLP-ST sensors, respectively. The detection limitsand limits of quantification were calculated to be 2.70×10^{-6} and 8.99×10^{-6}mol $L^{-1}$for CLP-TPB and $2.26\times10^{-6}$and 7.52×10^{-6}mol $L^{-1}$for CLP-ST sensors. The response time is about 10 s for both sensors.The selectivity studies showed that these sensors have higher selectivity towardCLP.HCl over a large number of cations and molecules. These sensors are successfully used for estimation of CLP.HCl in pharmaceutical formulation. The suggested method was validated by its comparison with the pharmacopeial one using t- and F-tests. The repeatability, reproducibility, ruggedness and robustness of the proposed methods were studied.

Keywords: Clomipramine hydrochloride. Chemically modified carbon paste sensor.

INTRODUCTION

Clomipramine hydrochloride (CLP.HCl),3-(3-chloro-10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-N,N-dimethylpropan-1-amine hydrochloride, molar mass 351.3 g mol^{-1}, scheme 1, is a tricyclic antidepressant[1] belonging to dibenzazepine class of drugs. It is a psychiatric medication used to treat and relief symptoms of depressive and obsessive-compulsive disorders[2] by inhibiting the reuptake of serotonin[3] by blocking its transporters, serotonin isneureotransmitter present naturallyin the brain and it is needed to maintain mental balance.



Scheme 1: Chemical structure of clomipramine hydrochloride

Several analytical methods have been reported for the determination of CLP.HCl in pharmaceutical formulations and biological fluids including spectrophotometric[4,5],chemiluminescence [6,7],spectrofluorimetric[8,9], capillary zone electrophoresis[10,11], high-performance liquid chromatography[12,13], gas chromatography[14,15],liquid chromatography-mass spectrometry[16], gas chromatography-mass spectrometry[17]. Most of these methods require the use of relatively costly sophisticated apparatus and complicated pre-treatment procedures like extraction of the active component. These requirements make it difficult for such methods to be used in routine analysis of large number of samples. As a result, suggesting of electrochemical methods of analysis using ion-selective sensors is an attractive alternative method for organic and inorganic detection, due to its advantages of being simple, rapid, reliable, low cost and non-destructive. Revealing the literature review, few potentiometric methods were found for determination of CLP.HCl[18,19]. Hence, the present work aims to develop twochemically modified carbon paste sensors for determination of CLP.HCl and to study their performance characteristics and their appliciability in potentiometric determination of CLP.HCl in pure solution, pharmaceutical formulations. These sensors are based on incorporation ofCLP-TPB and CLP-ST in spectroscopic graphite powder plasticized with o-nitrophenyl phenyl ether (o-NPPE) and o-nitrophenyloctyl ether (o-NPOE), respectively.

MATERIALS AND METHODS

Reagents and materials

All reagents and chemicals used were of analytical grade purity and all solutions were prepared in bidistilled water. ClompramineHCl and its pharmaceutical formulations (Anafronil tablets, 25 and 50 mg/tablet) were obtained from NOVARTIS PHARMA S.A.E. Cairo, Egypt. Sodium tetraphenylborate (NaTPB) $Na[C_{24}H_{20}B]$, silicotungestic acid (STA) ($H_4[W_{12}SiO_{40}]$), dibutyl phthalate (DBP), dioctyl phthalate (DOP), tricresyl phosphate (TCP), ethylhexyladipate (EHA), o-nitrophenyl phenyl ether (ONPPE), ethylhexylsebacate (EHS), o-nitrophenyloctyl ether (ONPOE), dioctylsebacate (DOS) and graphite powder were obtained from Sigma-Aldrich, USA.

0.1 mol L^{-1}solution of CLP.HCl was prepared by dissolving 3.513 g in 100 cm^3bidistilled water. The working standard solutions (1.00×10^{-7}-1.00×10^{-2}mol L^{-1}) were prepared by proper dilution of the stock solution with bidistilled water. A 1.00×10^{-2}mol L^{-1}NaTPB standard solution was prepared by dissolving 0.3422 g into 100 mL bi distilled water. Solutions of sodium hydroxide and hydrochloric acid of concentrations within the range 0.1-1.0 mol L^{-1}were used for adjusting the pH of the medium. The stock solutions and the dilutions were kept in dark brown bottles in the refrigerator.

To investigate the selectivity of the proposed electrodes, 0.1 mol L^{-1}chloride solutions of Na^+, K^+, NH_4^+, Ca^{2+}, Cu^{2+}, Mg^{2+}, nitrate solution of Fe^{3+} (obtained from Adwic chemical company, Abu Zabal, Egypt), sucrose, lactose, DL-histidine, L-cysteine, DL-asparagine, L-threonine, D-alanine and DL-serine (obtained from Aldrich chemical company) were prepared.

Apparatus

The potentiometric and pH measurements were carried out with a Jenway 3010 digital pH/mV meter. A techne circulator thermostat Model C-100 (Cambridge, England) was used to control the temperature of the test solution. A saturated calomel electrode (SCE) was used as the external reference. The electrochemical system of the CLP-CMCPS would be represented as CMCPS|testsolution|SCE. The elemental analysis of the prepared ion-exchangers was performed using automatic CHN analyzer (Perkin-Elmer model 2400) at the Micro-Analytical Center, Faculty of Science, Cairo University.

Preparation of the ion-exchangers

CLP-TPB and CLP-ST were prepared by mixing 50 mL of 1.00×10^{-2}mol L^{-1}CLP.HCl with 50 mL of 1.00×10^{-2}mol L^{-1}NaTPB or 0.25×10^{-2}mol L^{-1}STA. The resulting precipitates were left in contact with their mother liquor overnight to assure complete coagulation. The precipitates were then filtered, washed thoroughly with distilled water, dried at room temperature and then ground to fine powder to be used in the construction of the sensors.

Preperation of the sensors

The modified paste was prepared by mixing various amounts of CLP-TPB or CLP-ST (1-10%, w/w) and an appropriate amount of spectroscopic graphite powder (diameter, 1-2 µm) with plasticizer (ratio of graphite powder to plasticizer was 1:1, w/w). The mixture was carefully homogenized using agate pestle in agate mortar. After homogenization of the mixture, the paste was moved to a hole (7 mm diameter and 3.5 mm depth) at one end of a teflon holder (12 cm) and to the other end a stainless steel rod was inserted through the center of the holder to make electrical contact. This rod can move

up and down by screw movement to press the paste down when renewal of the electrode surface is needed. The external surface of the carbon paste was smoothed with soft paper.

Construction of calibrations graphs

Suitable increments of standard drug solution were added to 50 mLbidistilled water so as to cover the concentration range 1.00×10^{-7}-1.00×10^{-2}mol L^{-1}. The working sensor and reference sensors were immersed in the solution and the emf values were recorded at 25±1.0 ºC, after each addition. The recorded values were plotted versus -log [CLP.HCl].

Effect of pH

The effect of pH of the drug solution on the cell emf values in concentrations, 1.00×10^{-4}, 5.00×10^{-4} and 1.00×10^{-3} mol $L^{-1}$was studied. Aliquots of drug solution (50 mL) were transferred to the titration cell and the tested sensor in conjunction with a saturated calomel electrode was immersed in this solution. The emf and pH readings were simultaneously recorded. The pH of the solution was varied over range of 1.0-12.0 by addition of very small volumes of 1.0 mol L^{-1}HCl or 0.1-1.0 mol L^{-1}NaOH solutions. The emf readings were plotted against the pH for the different drug concentrations.

Effect of temperature

To study the temperature effect of the sensors, calibration graphs were constructed at different test solution temperatures (t) covering the range of 25-55 ºC with the aid of a circular thermostat Model C-100 (Cambrige, England). The slope, the standard sensor potential (E^o_{sen}), usable concentration ranges and response time of the sensor corresponding to each temperature were calculated in this temperature range. For the determination of the temperature coefficients of the sensors, the values of Eº were plotted versus (t-25). The slope of the straight line obtained represents the thermal coefficient (dEº/dt) which was calculated for each sensor using the following equation[25]:

$$E^o_{cell}=E^o_{25^oC} + (dE^o/dt)\ (t-25)$$

Plot of Eºcell 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^o_{sen}) were calculated after subtraction of the potential of the calomel electrode at different temperatures.

Effect of interfering ions

The selectivity coefficient values were calculated by applying the matched potential method[26]. The matched potential is unique because it depends on neither the Nicolsky-Eisenman equation nor any of its modifications. In this method, the potentiometric selectivity coefficient is defined as the activity ratio of primary and interfering ions that give the same potential change under identical conditions. The activity of the drug was increased from a_{drug} =1.00×10^{-5}mol L^{-1}(reference solution) to $\bar{a}_{drug}$ by adding 0.10 mL of 1.00×10^{-2}mol $L^{-1}$of drug solution, and the change in potential ΔE corresponding to this increase in activity is measured. Next, a solution of interfering ion of concentration a_j in the range of (1.00×10^{-3}-1.00×10^{-1}mol L^{-1}) was added to new 1.00×10^{-5}mol L^{-1}drug reference solution until the same potential change (ΔE) is recorded. The selectivity coefficient values $K^{pot}_{dru_j}$ were then calculated using the following equation[26]:

$$K^{pot}_{drug,j^{z+}} = (a_{drug})/(a_j)$$

Where a_j: the activity of the added interferent

In case of cations that cause interference, the mixed solution method[27] was carried out to detect the extent of interference of different ions. This was carried out by measuring the emf in presence of 1.0, 5.0, 10.0, 100, 1000 µmol $L^{-1}$of the interfering ion and they were plotted against -log [CLP.HCl].

Stiochiometric ratio using conductimetric measurement

1.00×10^{-3} mol $L^{-1}$of the drug, 1.00×10^{-2}mol L^{-1}NaTPB and STA were prepared. 50 mL of the prepared CLP.HCl solution were transferred to the conductivity cell. Then 1.00×10^{-2}mol $L^{-1}$of NaTPB or STA was added and the conductance was measured at 25 ºC subsequent to each addition of the reagent after thorough stirring. The conductance reading after each addition was corrected for dilution by means of the following equation[28]:

$$\Omega_{corr}=\Omega_{obs}\ [(V_1+V_2)/V_1]$$

Where, Ω is the electrolytic conductivity (corr., corrected; obs., observed), V_1 is the initial volume and V_2 is the volume of the added titrant.
The conductimetric titration graphs were constructed by plotting the volume (mL) of the titrant added against corrected conductance (μS cm^{-1}) and the end point was determined. The molar ratio was calculated for each increment of CLP.HCl and plotted against the conductance values.

Standard addition method
The standard addition method was applied, in which small increments of standard CLP.HCl solution were added to 50 mL aliquot of samples of various concentrations (1.00×10^{-7}-1.00×10^{-2}mol L^{-1}) of pure drug, its pharmaceutical preparation (tablets). The change in potential reading was recorded for each increment and used to calculate the concentration of the drug in sample solution using the following equation[29]:

$$C_x = C_s \left(\frac{V_S}{V_X + V_S} \right) \left[10^{n(\Delta E/S)} - \frac{V_X}{V_S + V_X} \right]^{-1}$$

Where C_x is the concentration to be determined, V_x is the volume of the original sample solution, V_s and C_s are the volume and concentration of standard solution added to the sample to be analyzed, respectively, ΔE is the change in potential after addition of a certain volume of standard solution and s is the slope of the calibration graph.

Potentiometric titration
Different aliquots of the investigated drug solution (1.00×10^{-2}mol L^{-1}CLP.HCL) were transferred into 100 mL titration cell and diluted to 50 mL with bidistilled water and the resulting solutions were titrated against 1.00×10^{-3} or 1.00×10^{-2}mol L^{-1}NaTPB solution. The emf values were recorded against the volume of the titrant added (V) and plotted as E vs V graph. The end points were determined from the conventional S-shaped curves and by the first derivative. The same procedure was applied for tablet.

Analysis of tablets
10-Tablets were accurately weighed and ground to fine powder in mortar and appropriate weight from this powder was taken and dissolved in 30 mL of bidistilled water and the mixture was filtered in 50 mL measuring flask. The residue was washed three times with bidistilled water and the volume was completed to the mark using the same solvent. After that, the standard addition method and potentiometric titration were applied.

RESULTS AND DISCUSSION

Sensors characterization
Different carbon paste sensors were prepared by varying the percentages of the ion-exchanger (CLP-TPB and CLP-ST) and using different solvent mediators to obtain theoptimum compositions of the sensors which give the best performance characteristics Table 1. The solvent mediator acts as a fluidizer allowing homogenous dissolution and diffusion mobility of the ion-exchanger inside the paste. For each composition, thetrial was repeated four times and the preparation process was highly reproducible as revealed by the low RSD% values of the obtained slopes.

Table 1: Response characteristics of the CLP-TPB and CLP-ST carbon paste sensors, average of 4 replicates at 25±1 ºC

Composition (w/w) %			Slope	Linear range	LOD	RSD*	r^2
Ion-exchanger	G.	Solvent mediator	mV $decade^{-1}$	mol L^{-1}		%	
CLP-TPB							
1	49.5	49.5 DBP	54.29±0.06	7.93×10^{-6}-1.00×10^{-2}	3.67×10^{-6}	0.20	0.9997
2	49.0	49.0 DBP	55.01±0.29	3.98×10^{-6}-1.00×10^{-2}	2.65×10^{-6}	0.92	0.9991
3	48.5	48.5 DBP	52.36±0.25	5.96×10^{-6}-1.00×10^{-2}	4.27×10^{-6}	0.85	0.9993
5	47.5	47.5 DBP	50.98±0.40	1.96×10^{-5}-1.00×10^{-2}	1.00×10^{-5}	1.36	0.9990
2	49.0	49.0 TCP	50.37±0.05	1.99×10^{-6}-1.00×10^{-2}	1.47×10^{-6}	0.18	0.9979
2	49.0	49.0 o-NPOE	55.85±0.53	7.93×10^{-6}-5.00×10^{-3}	4.62×10^{-6}	1.64	0.9992
2[a]	49.0	49.0 o-NPPE	60.53±0.14	3.74×10^{-6}-1.00×10^{-2}	2.70×10^{-6}	0.41	0.9991
2	49.0	49.0 DOP	42.51±0.35	3.98×10^{-6}-1.00×10^{-2}	2.67×10^{-6}	1.43	0.9969
CLP-ST							
1	49.5	49.5 DBP	43.32±0.38	1.99×10^{-6}-1.00×10^{-2}	1.18×10^{-6}	1.54	0.9980
2	49.0	49.0 DBP	50.23±0.21	3.36×10^{-6}-1.00×10^{-2}	2.12×10^{-6}	1.85	0.9994
3	48.5	48.5 DBP	54.51±0.36	3.21×10^{-6}-1.00×10^{-2}	2.67×10^{-6}	1.15	0.9995

5	47.5	47.5 DBP	55.36±0.10	3.07×10^{-6}-1.00×10^{-2}	2.43×10^{-6}	0.33	0.9979
7	46.5	46.5 DBP	57.02±0.08	3.98×10^{-6}-1.00×10^{-2}	3.35×10^{-6}	0.26	0.9991
10	45.0	45.0 DBP	54.70±0.08	7.93×10^{-6}-1.00×10^{-2}	4.93×10^{-6}	0.28	0.9978
7[b]	46.5	46.5 o-NPOE	59.22±0.12	3.98×10^{-6}-1.00×10^{-2}	2.26×10^{-6}	0.36	0.9994
7	46.5	46.5EHA	45.49±0.16	5.65×10^{-6}-1.00×10^{-2}	4.36×10^{-5}	0.61	0.9995
7	46.5	46.5 TCP	42.83±0.68	3.98×10^{-6}-1.00×10^{-2}	2.15×10^{-6}	1.96	0.9745
7	46.5	46.5 DOP	30.95±0.31	1.19×10^{-6}-1.00×10^{-2}	9.82×10^{-7}	1.89	0.9926
7	46.5	46.5 DOS	24.61±0.14	2.36×10^{-6}-1.00×10^{-2}	1.08×10^{-6}	1.02	0.9957

a and b: Selected CMCPSs, *RSD of four replicate measurements, r^2: correlation coefficient, G: graphite powder

The results show that the sensors with composition 2% CLP-TPB, 49% o-NPPE and 49% graphite powder and 7% CLP-ST, 46.5% o-NPOE and 46.5% graphite powder exhibit the best performance characteristics, assigned with (a,b) in Table 1. The sensors show Nernstain slopes of 60.53±0.14 and 59.22±0.12 mV decade^{-1}in concentration ranges of 3.74×10^{-6}-1.00×10^{-2} and 3.98×10^{-6}-1.00×10^{-2}mol $L^{-1}$for CLP-TPB and CLP-ST, respectively. Thelimits of detection (LODs) were calculated to be 2.70×10^{-6} and 2.26×10^{-6}mol $L^{-1}$and limits of quantification (LOQs) were8.99×10^{-6} and 7.52×10^{-6}mol $L^{-1}$for CLP-TPB and CLP-ST, respectively(Figure 1). The sensors of the optimum composition were used directly to carry out all the subsequent studies without any soaking.

Stoichiometry of the ion-exchangers

Stoichiometries of the ion-exchangers were found to be 1:1 and 1:4 for CLP-TPB and CLP-ST, respectively. For CLP-TPB, the C, H and N percentages are 79.90,6.73and4.38% and the corresponding calculated ones are79.10, 6.95,and 4.29%. For CLP-ST, the C, H and N percentages are 22.30, 2.36 and2.75% and the corresponding calculated ones are 22.08, 2.24, and 2.71%. These data wereconfirmed using conductimetric titration.

Life time

The life span of the sensors is closely related to the nature of the solvent mediator and the ion-exchangers and its rate of leaching to the solutions [20]. Life times were examined by measuring the slope of the calibration graphs for variable intervals of time starting from 2 h reaching to 3 months. The results showed that the life span (t) is more than 90 days for CLP-TPB and 30 days for CLP-ST sensors.

Effect of pH

The influence of the solution pH on the sensor response was checked for three concentrations of CLP.HCl (1.00×10^{-3}, 5.00×10^{-4} and 1.00×10^{-4}mol L^{-1}) by measuring the variation in emf values with change in the solution pH by addition of very small volumes of hydrochloric acid and sodium hydroxide (each 0.1-1.0mol L^{-1}). The results indicate that, the sensors show no response to the pH change in the range of 2.00-7.57 and 2.10-7.34 for CLP-TPB and CLP-ST sensors, respectively (Figure2). At higher pH the potential decreases gradually and this can probably be attributed to the formation of the free base of the CLP in the solutions leading to decrease in the concentration of CLP^+ [21].

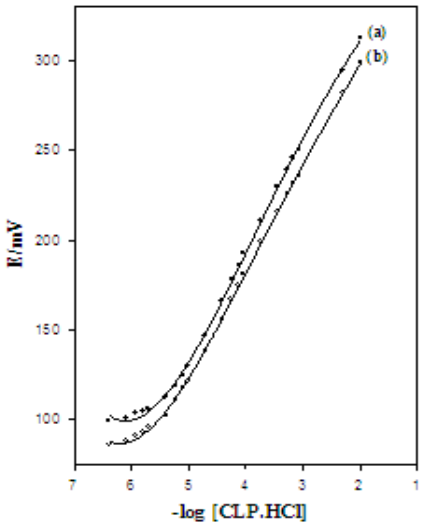


Fig. 1: Calibration curves of CLP-TPB (a) and CLP-ST CMCPSs (b) at 25±1.0ºC

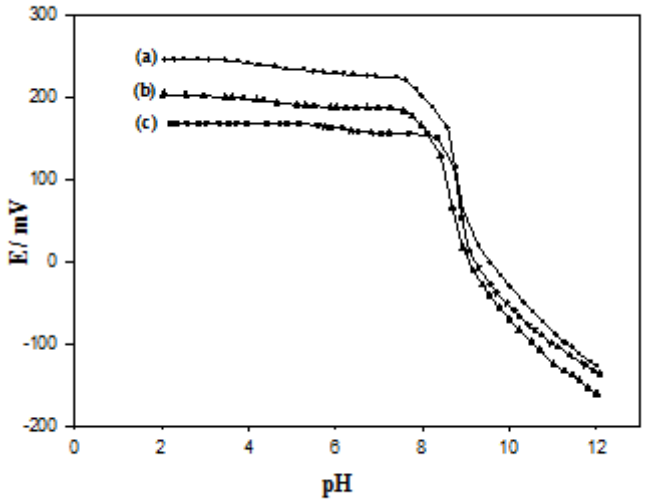


Fig. 2: Effect of pH on 1×10-3 (a), 5×10-4 (b) and 1×10-4 M CLP.HCl (c) solutions on the potential response of CLP-TPB CMCPS.

Response time

The response time[22] of the proposed sensors was tested by measuring the time required to achieve a steady state potential (within±1 mV) after successive immersion of the sensor in a series of drug solutions each having a 10-fold increase in concentration from 1.00×10^{-5} to 1.00×10^{-2}mol L^{-1}. The sensor showed steady state potentials within 5-10 s. The emf stay constant (within±1 mV) for at least 4 min. Typical potential-time plots for the response characteristics of the CLP- TPB and CLP-ST sensors were shown in (Figure 3). When the sensors were transferred from 1.0×10^{-4} to 1.0×10^{-5}mol/L solutions, it was stabilized to values higher than the calculated ones, which may be due to memory effect.

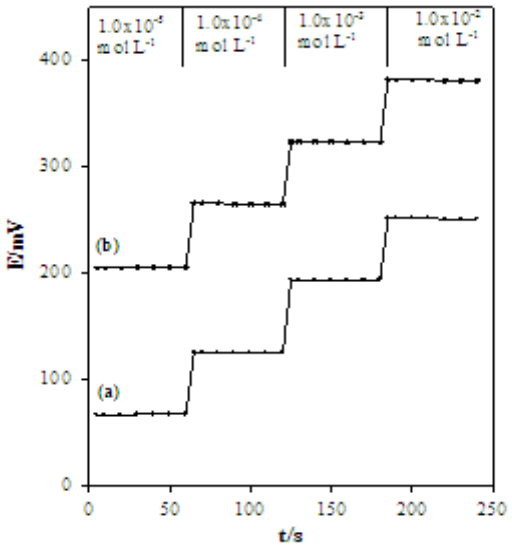


Fig. 3: Potential-time plot for the the response of CLP-TPB (a) and CLP-ST CMCPSs (b)

Effect of temperature

For the determination of the temperature coefficients of the sensors, the values of Eº were plotted versus (t-25). The slopes of the straight lines obtained represent the temperature coefficients of the cells(Figure4)amounting to -0.4280 and -0.3765 V/ºCand that of the sensors were calculated to be -1.61 and -1.24V/ºC for CLP-TPB and CLP-ST,respectively. The small values of reveal a thermal stability of the cell emf within the temperature range investigated.

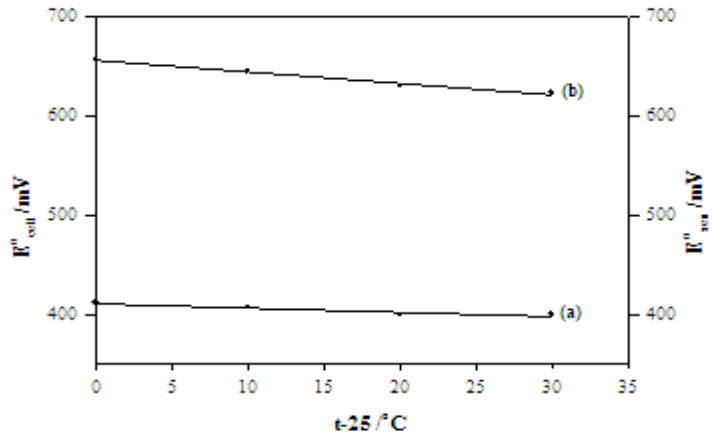


Fig. 4: Variation of the cell standard potential (a) and the the sensor standard potential (b) of CLP-TPB CMCPS with change of test solution temperature

Response to other ions

The influence of some inorganic cations, sugars and amino acids, which may have been present in pharmaceutical preparations as excipients or additives, were investigated. The obtained selectivity coefficients are very small values except for Fe(III) and Cu(II) as shown in Table2 and this indicates a very high selectivity of the prepared sensors towardCLP.HCl. Inorganic cations do not interfere because of the differences in ionic size, mobility and permeability compared with CLP^+. The high selectivity over amino acids can be attributed to the differences in polarity and lipophilic nature of their molecules relative to CLP ion.

Table 2: Selectivity coefficient (-log K^{pot}_{drug} of CLP-TPB and CLP-ST CMCPSs by the matched potential method

Interferent	CLP-TPB	CLP-ST
Na^+	2.88	2.92
K^+	2.90	2.72
NH_4^+	2.21	3.13
Ca^{2+}	2.85	2.65
Mg^{2+}	2.14	1.90
Cu^{2+}	0.77	0.69
Fe^{3+}	0.30	0.17
Sucrose	3.06	3.30
Lactose	3.47	2.73
DL-Histidine	1.17	1.17
L-cysteine	1.77	1.30
DL-Asparagine	2.42	1.47
L-threonine	2.90	2.54
D-Alanine	2.83	2.63
DL-serine	2.87	2.90

The selectivity coefficients for Fe(III) and Cu(II)ions are very small, and it cause some interference if presentin high concentrations. To estimate the extent of interferencecaused by the Fe(III) and Cu(II) ions, mixed run studies were performed, andthe effect of the Fe(III) and Cu(II)concentration on the performance of thesesensors was examined. The potentials were measured in thepresence of different concentrations (1.0, 5.0, 10.0, 100, 1000 µmol L^{-1}) of Fe(III) and Cu(II)ions. The results indicate that the sensors show no significant interference at the lower concentrations (up to 5 µmol L^{-1}) of Fe(III) and Cu(II) ions, and the sensors can be used for the estimation of CLP.HCl, However, in the presence of high concentrations of Fe(III) and Cu(II)ions concentrations (above 5 µmol L^{-1}), significant interference was observed, which caused a shortening of the linear range with a high detection limit (Figure5). Interference of Fe(III) and Cu(II)can be eliminated using 5 mL of 0.05 mol $L^{-1}$of EDTA. The effect of EDTA on the characteristics response of the calibration graphs was studied, and the result showed that there is small deviation in the slope, LOD and linear range values(Figure6).

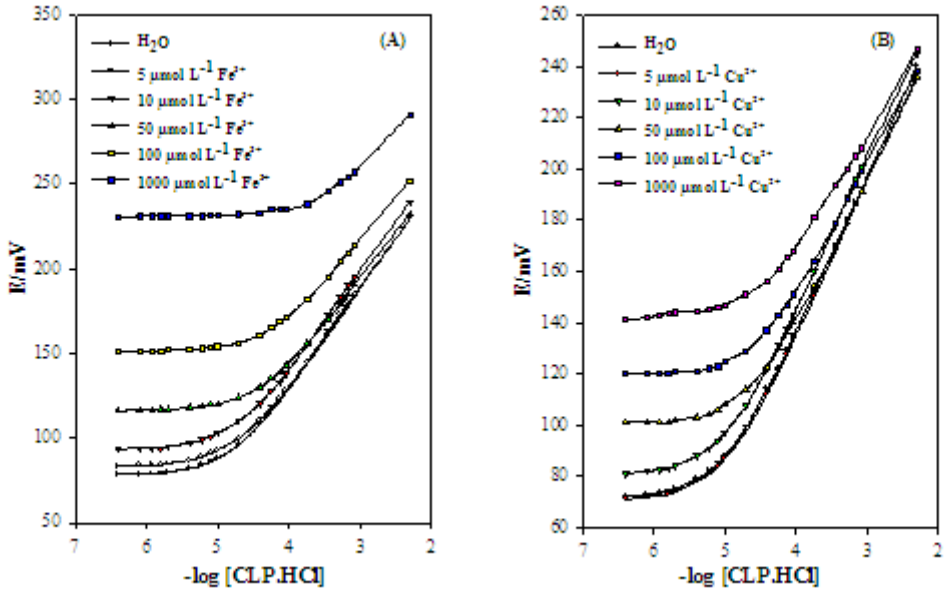


Fig. 5: Calibration graphs for CLP-TPB CMCPS at diffeent concentrations of iron (III) (A) and copper (II) (B)

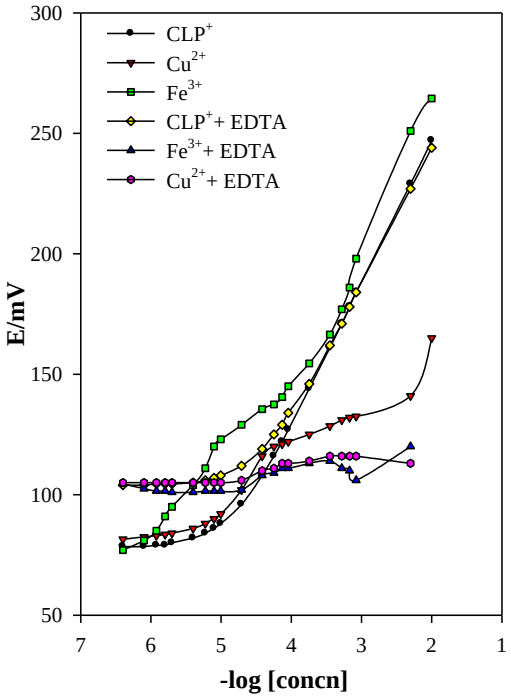


Fig. 6: Response to Fe(III) and Cu(II) in presence and absence of EDTA using CLP-TPB CMCPS

Analytical applications

The proposed sensors can be used for determination of CLP.HCl in pure solution andpharmaceutical formulationsusing the methods of potentiometric titration(Figure 7) and standard addition. The obtained LOD and LOQ using potentiometric titration are 5.99×10^{-7} and 1.99×10^{-6}mol L^{-1}. In case of standard addition method,the LOD and LOQ are 3.00×10^{-8} and 9.99×10^{-8}mol L^{-1}. The mean recovery and the relative standard deviation values are summarized in Tables3. The data indicate that there is no interference from excipients used in tablets.

Table 3: Determination of CLP. HCl in its pure solution and pharmaceutical formulation applying the potentiometric titration and the standard addition method using CLP-TPB and CLP-ST CMCPSs

Potentiometric titration					Standard addition method				
	CLP-TPB/o-NPPE		CLP-ST/o-NPOE			CLP-TPB/o-NPPE		CLP-ST/o-NPOE	
Taken	Recovery	RSD*	Recovery	RSD*	Taken	Recovery	RSD*	Recovery	RSD*
(mg)	(%)				(mg)	(%)			
Pure solution									
1.05	104.58±0.16	0.30	102.07±0.41	0.81	0.0017	99.75±0.42	0.84	101.49±0.41	0.81
1.75	103.50±0.28	0.55	101.25±0.47	0.94	0.0175	103.70±0.33	0.63	101.06±0.49	0.98
3.51	102.50±0.28	0.56	102.59±0.29	0.57	0.1750	101.80±0.75	1.28	101.67±0.16	0.33
10.53	100.33±0.19	0.38	102.49±0.48	0.94	1.7500	101.56±0.03	0.56	101.30±0.39	0.78
17.56	100.40±0.28	0.56	101.40±0.47	0.93					
35.13	99.72±0.13	0.26	100.42±0.21	0.43					
Anafronil tablet (25 mg/tablet)									
1.49	100.83±0.39	0.78	100.37±0.23	0.47	0.1700	103.24±0.29	0.57	102.15±0.43	0.84
2.49	101.85±0.29	0.57	99.75±0.14	0.28	1.7500	102.41±0.30	0.59	100.38±0.34	0.69
Anafronil tablet (75 mg/tablet)									
4.49	99.66±0.23	0.47	99.65±0.19	0.38	0.1700	101.01±0.72	1.43	102.35±0.34	0.66
7.49	98.50±0.50	1.02	98.50±0.28	0.58	1.7500	101.36±0.11	0.22	101.08±0.36	0.71

* Relative standard deviation of four repetitive measurements.

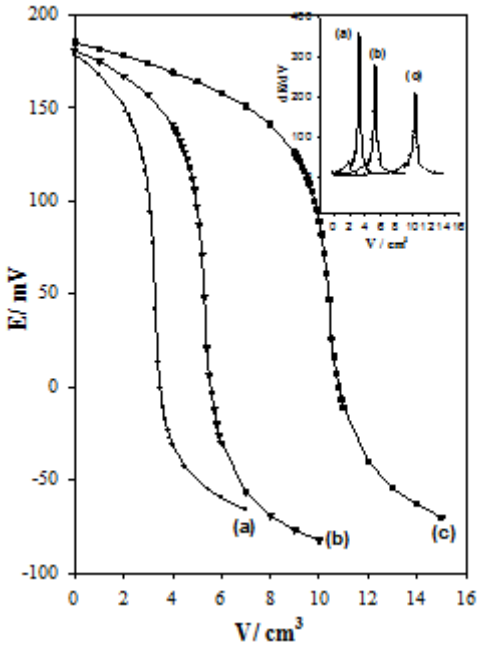


Fig. 7: Potentiometric titration of 1.05 (a), 1.75 (b) and 3.51 mg CLP.HCl (c) against 1.00×10-3 M NaTPB using CLP-TPB CMCPS

Validation of the methods

Repeatability and reproducibility

The studied sensors were tested for their repeatability and reproducibility study of their characteristics carrying out the calibration curves four times for the same sensor in case of repeatability study. In case of reproducibility, the sensor of the same composition was prepared four times and the calibration curves were constructed.For repeatability of the sensors it was found that the slope values were 60.47±0.56 and 59.51±0.47 mV decade^{-1}with RSD% 0.86 and 1.59% for CLP-TPB and CLP-ST sensors, respectively. For reproducibility of the sensors it was found that the slope values were 59.97±0.39and 59.05±0.06 mV decade^{-1}with RSD% 1.33 and 0.20% for CLP-TPB and CLP-ST CMCPSs, respectively.Four replicates determination at different concentration levels are carried out using both CMCPSs to test the precision of the method Table 3. The RSD% values were found to be less than 1.5, indicating reasonable repeatability and reproducibility of the proposed method.

Statistical treatment of the obtained data

The results were statistically compared with the pharmacopeia method [1]using t- and F-tests, Table4. At 95% confidence level for 4 replicate measurements, the calculated t- and F-values did not exceed the critical values, indicating that there is no significant difference between the proposed method and the official method with regard to accuracy and precision.

Table 4: Statistical treatment of the obtained data for the determination of CLP.HCl applying the potentiometric titration and the standard addition method using CLP-TPB and CLP-ST CMCPSs in comparsion with official method

Official method		CLP-TPB			CLP-ST		
		Pure solution	Anafronil tablet (25 mg/tablet)	Anafronil tablet (75 mg/tablet)	Pure solution	Anafronil tablet (25 mg/tablet)	Anafronil tablet (75 mg/tablet)
Potentiometric titration							
X±S.E	**101.59±0.72**	**103.31±0.26**	**101.30±0.47**	**99.75±0.85**	**102.25±0.47**	**99.75±0.14**	**98.50±0.28**
RSD%*	1.42	0.50	0.93	1.71	0.94	0.28	0.58
t-value		1.64	0.85	1.56	1.63	2.84	3.53
F-value		0.12	0.42	1.39	0.43	0.04	0.15
Standard addition method							
X±S.E	**101.59±0.72**	**101.68±0.86**	**102.41±0.30**	**101.36±0.11**	**101.67±0.16**	**102.15±0.43**	**102.35±0.34**
RSD%*	1.42	1.48	0.59	0.22	0.33	0.84	0.66
t-test		0.53	0.45	0.72	0.39	0.17	0.42
F-test		1.08	0.18	0.03	0.05	0.36	0.32

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

Ruggedness and robustness of the proposed methods

For ruggedness of the methods, a comparison between the results obtained by two analysts was performed. The RSD% values obtained by two analysts in the same laboratory are 0.99and 0.79% for CLP-TPB and 0.72 and 0.99% for CLP-ST. The robustness of the proposed methods was studied while the temperature of the solution wasslightly changed. The recovery percentages were good under most conditions and do not show any significant change when the critical parameter were modified.

CONCLUSION

The suggested sensors were characterized to obtain the best composition and the best conditions for constructing the calibration curves. The sensors exhibit Nernstian response with slope 60.53±0.14 and 59.22±0.12 mV decade^{-1}in the concentration range of 3.74×10^{-6}-1.00×10^{-2} and 3.98×10^{-6}-1.00×10^{-2}mol L^{-1}over pH range of 2.10-7.57 and 2.00-7.34, for Ser-TPB and Ser-ST CMCPSs, respectively, with response time less than 10 s. LOD and LOQ are 2.70×10^{-6} and 2.26×10^{-6} and 8.99×10^{-6} and 7.52×10^{-6}mol L^{-1}for CLP-TPB and CLP-ST sensors, respectively. The selectivity studies revealed that the prepared sensors have high selectivity toward CLP^+ over a wide range of other cations and molecules except for Fe(III) and Cu(II) which can be masked by EDTA masking technique. These sensors were successfully applied for the determination of CLP^+ in pure samples andAnafronil tablets. The obtained results are in good agreement with those obtained from the British pharmacopeial one. The obtained data were validated and compared with the other reported ones, Table 5. The results show that the suggested sensors have a wide linearity range, Nernastian slope, low LOD, LOQ valuesand long life time, Table 5. They are also repeatable and reproducible sensors.

Table 5: Comparison between the suggested and some of the other published electrochemical methods for determination of clomipramine hydrochloride

Reference	Composition	Slope	Linear range	LOD	t_{res}	Life span	Working pH range	r^2
	(W/W)	mV decade^{-1}	mol L^{-1}		s			
CMCPS								
Present work	2%CLP-TPB + 49% graphite powder + 49% o-NPPE	60.53±0.14	3.74×10^{-6}-1.00×10^{-2}	2.70×10^{-6}	<10	More than 3 months	2.10-7.61	0.9991
Present work	7% + CLP-ST + 46.5% graphite powder + 46.5% o-NPOE	59.22±0.12	3.98×10^{-6}-1.00×10^{-2}	2.26×10^{-6}	<10	one month	2.00-7.35	0.9994

[19]	25% ion-pair + 5% MWCNTS + 25% ionic liquid + 45% graphite powder	58.50±0.30	1.00×10^{-5}-1.00×10^{-2}	1.00×10^{-5}	20	8 weeks	3.00-6.50	0.9970
Membrane sensors								
[19]	7% ion-pair + 30%PVC + 63% DBP	58.20±0.50	1.00×10^{-4}-1.00×10^{-2}	8.50×10^{-5}	25	5 weeks	3.00-6.50	0.9960
[28]	CLP-Rein + DOS + PVC	52.28±0.15	1.00×10^{-7}-1.00×10^{-2}	1.00×10^{-7}	20	6 weeks	2.00-5.00	0.9950
[28]	CLP-Rein + DOP + PVC	52.00±0.21	1.00×10^{-7}-1.00×10^{-2}	1.00×10^{-7}	25	6 weeks	2.00-5.00	0.9950
[29]	1% TBA-TPB + 66% PVC + 33% NPOE	57.50±0.95	1.00×10^{-5}-1.00×10^{-2}	$(3\pm0.7)\times10^{-6}$	<13	More than 2 months	3.00-5.50	------

t_{res}: response time, r^2: correlation coefficient

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