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POLYMERIC MEMBRANE SENSORS FOR DIRECT DETERMINATION OF TRICYCLIC ANTIDEPRESSANT CLOMIPRAMINE HYDROCHLORIDE IN PHARMACEUTICAL FORMULATIONS AND BIOLOGICAL FLUIDS

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ABSTRACT

The construction and performance characteristics of clomipramine hydrochloride selective electrodes were developed. Three types of electrodes: plastic membrane I, coated wire II, and coated graphite III were constructed based on the incorporation of clomipramine hydrochloride with ammonium reineckate. The influence of membrane composition, kind of plasticizer, pH of the test solution, soaking time, and foreign ions on the electrodes was investigated. The electrodes showed Nernstain responses with mean calibration graph slopes of 58.90±0.2, 59.33±0.1 and 56.07±0.1 mV decade^{-1} at 25°C for electrodes I, II and III, respectively over clomipramine concentration ranges from 1.0x10^{-2}-5.0 x10^{-6}, 1.0x10^{-2}-1.0x10^{-6} and 1.0x10^{-2}-1.0 x10^{-5} mol L^{-1} with detection limits 1.9x10^{-6}, 4.8x10^{-7} and 5.0x10^{-6} mol L^{-1} for electrodes I, II and III, respectively. The constructed electrodes gave average selective precise and sensitivity within the pH range 4-8. Interferences from common cations, alkaloids, sugars, amino acids and drug excipients were reported. The proposed electrodes were also successfully applied to the determination of the drug in pharmaceutical preparations and biological fluids.

Keywords: Plastic membrane; Coated wire electrode; Coated graphite rod; Ion selective electrode; Clomipramine hydrochlorides; Potentiometric determination.

INTRODUCTION

Clomipramine hydrochloride, 3-(3-chloro-10,11-dihydro-5*H*-dibenzo{*b,1*}azepin-5-yl)-N - N - dimethyl propan -1-amine hydrochloride (Figure 1) is a typical tricyclic anti-depressant with a wide clinical spectrum being used in major depressive, panic and obsessive-compulsive disorders [1]. The therapeutic and pharmacologic importance of clomipramine has prompted the development of several methods for its determination, both in body fluids and pharmaceuticals, including high performance liquid chromatography [2-4], liquid chromatography coupled with mass spectrometry [5-7], gas chromatography [8], gas chromatography coupled with mass spectrometry [9], capillary electrophoresis [10,11], spectrophotometry [12] , Conductimetry [13], Spectrofluorimetry [14,15], potentiomerty [16].

This present study aims to develop new selective membrane sensors, of three types: plastic membrane, coated wire and coated graphite electrodes for the determination of clomipramine hydrochloride in pure solutions, pharmaceutical preparations and biological fluids.

EXPERIMENTAL

Equipment

Jenway 3040 pH/mV meter (U.K.) with a clomipramine-PVC membrane electrode type (I), clomipramine-coated wire electrode type (II) or clomipramine-coated graphite rod electrode type (III) in

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conjunction with double junction Ag/AgCl electrode (Orion 90-02) (Taiwan, R.O.C.) containing 10%w/v potassium nitrate in outer compartment. An Orion 91-02 magnetic stirring.

Reagents

All chemicals used were analytical or pharmacopoeial grade. Doubly distilled water was used throughout the experiments. Clomipramine hydrochloride was provided by Novartis, poly(vinyl chloride) PVC powder with high molecular weight was from Aldrich (Germany), dibutyl phthalate (DBP), dioctyl phthalate (DOP), dinonyl phathalate (DNP), were from Fluka (Buchs, Switzerland), ammonium reineckate, chloroform, acetone, hydrochloric acid and tetrahydrofuran (THF) were from Memphis-Delagrange (France), (Supranil® capsules, Anafranil® tablets) were purchased from local drug stores. Stock clomipramine hydrochloride solution (1.0×10^{-1} mol L^{-1}) was prepared daily by dissolving an appropriate amount of the drug in double distilled water. More dilute solutions were prepared by appropriate dilution.

Preparation of clomipramine reineckate ion-pair

The ion-pair was prepared by mixing stoichiometric amounts of 1.0×10^{-2} mol L^{-1} ammonium reineckate with an equimolar solution of clomipramine hydrochloride, stirred for 10 min. The resulting pink precipitate was filtered through G_4 sintered glass crucible and washed thoroughly with deionized water then dried at room temperature for 24 hours. The ion-pair should be stored in a desiccator.

Membrane composition

The membrane composition was studied by varying the percentages (w/w) of the ion pair, poly (vinyl chloride) PVC and plasticizer (DOP), until the optimum composition that exhibits the best performance characteristics was obtained. The membranes were prepared by dissolving the required amount of the ion-pair, PVC and (DOP), in 5 mL tetrahydrofuran (THF). The solution mixture was poured into a petri dish (3 cm in diameter), covered with a filter paper and the solvent was allowed to evaporate slowly at room temperature. To obtain the uniform membrane thickness, the amount of (THF) was kept constant, and its evaporation was fixed for 24h.

Electrode construction

Plastic membrane electrode

A punched circular membrane was attached to a poly-ethylene tube (8 mm in diameter) in an electrode configuration by means of PVC-THF solution. A mixture containing equal volume of 1.0×10^{-3} mol L^{-1} clomipramine hydrochloride and potassium chloride was used as internal reference solution in which the Ag/AgCl reference glass-calomel combination electrode, (Taiwan, R.O.C.) was used for pH adjustment. All potentiometric measurements were carried out at 25±1 ºC with constant electrode was dipped. The constructed electrode was pre-conditioned after preparation by soaking for at least 24 h in 1.0×10^{-3} mol L^{-1} clomipramine hydrochloride and stored in the same solution. All potentiometric measurements were performed using the following cell assembly: Ag/AgCl/Internal solution /membrane/test solution//KCl salt bridge//SCE.

Coated wire electrode

Pure aluminum wire of 4.0 cm length was tightly insulated by polyethylene tube leaving 1.0 cm at one end for the coating and 0.5 cm at the other end for connection. The coating solution was (described previously under membrane composition). Prior to coating, the polished aluminum surface was washed with a detergent and water, thoroughly rinsed with water, and dried with acetone.

Then the wire was rinsed with chloroform and allowed to dry. Afterwards, the aluminum wire was coated by quickly dipping it into the coating solution several times, and allowing the film left on the wire to dry for about 3 min. The process was repeated several times until a plastic membrane of approximately 1.0 mm thickness was formed [17]. The prepared electrode was conditioned by soaking for 3 h in 1.0×10^{-3} mol L^{-1} clomipramine hydrochloride solution. All potentiometric measurements were performed using the following cell assembly: Al/membrane/test solution//KCl salt bridge//SCE.

Coated graphite electrode

A pure graphite rod of 4 mm diameter was insulated by tight polyethylene tube, leaving 2 cm at one end for coating and 1 cm at the other end for connection. The polished electrode surface was coated with the active membrane by dipping the exposed end into the coating solution was (described previously under membrane composition) ten times and allowing the film left on graphite rod to dry in air for 1 min each time. The process was repeated until 1.0 mm thickness formed. The prepared electrode was preconditioned by soaking for 6 h in 1.0×10^{-3} mol L^{-1} clomipramine hydrochloride solution [18].

Electrode calibration

10 mL aliquots of 1.0×10^{-1}-1.0×10^{-6} mol L^{-1} standard clomipramine hydrochloride solutions were transferred into 50 mL beaker and the sensor(s) in conjunction with double junction Ag/AgCl reference electrode were immersed in the solution. The measured potential was plotted against the logarithm of drug concentration. The electrode(s) was washed with deionized water and dried with tissue paper between measurements.

Effect of pH

The effect of pH on the potential of the electrode(s) was measured using two pH/mV meters. The combined glass calomel electrode was connected to one instrument and the clomipramine-electrode(s) with the double junction Ag/AgCl reference electrode was connected to the second instrument. Thirty mL aliquots of $1.0x10^{-3}$ mol L^{-1}, drug solution were transferred to a 100 mL beaker where the electrodes were immersed, the potential readings corresponding to different pH values were recorded. The pH was gradually increased or decreased by addition of small aliquots of dilute solutions of 0.1N sodium hydroxide or 0.1N hydrochloric acid respectively, and the pH-mV was measured and plotted.

Sensor selectivity

Selectivity coefficients were determined by the separate solution method [19] in which the following equation was applied.

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

Where, E_1 is the electrode potential in $1.0x10^{-3}$ mol L^{-1} clomipramine hydrochloride solution. E_2 is the potential of the electrode in $1.0x10^{-3}$ mol L^{-1} solution of the interferent ion J^{z+} and S is the slope of the calibration plot. The selectivity of the electrode(s) towards sugars, amino acids, certain cations and alkaloids was studied.

Standard addition method

The fabricated electrode(s) was immersed into sample of 50 mL with unknown concentration (ca. $1.0x10^{-4}$ mol L^{-1}) and the equilibrium potential of E_1 was recorded. Then 0.1 mL of $1.0x10^{-1}$ mol L^{-1} of standard drug solution was added into the testing solution and the equilibrium potential of E_2 was obtained, from the change of ΔE (E_2-E_1) one can determine the concentration of the testing sample. The standard addition technique was used for the analysis of clomipramine tablets [20].

ANALYTICAL APPLICATIONS

Determination of clomipramine hydrochloride in tablets and capsules

Ten tablets were finely powdered shaken with 100 mL distilled water to obtain different concentrations in the range of $5.0x10^{-3}$-$1.0x10^{-5}$ mol L^{-1} for tablets and $1.0x10^{-2}$-$5.0x10^{-5}$ mol L^{-1} for capsules. The prepared solutions were adjusted to pH 4 using 0.1N dilute hydrochloric acid. The clomipramine-electrode(s) were immersed in the solution. The electrode(s) system was allowed to equilibrate with stirring and the e.m.f. was recorded and compared with the calibration graph.

Content uniformity assay of clomipramine capsules

Ten individual capsules of 50 mg/cap were placed in separate 100 mL beakers and dissolved in 90-100 mL of distilled water. The electrode(s) was directly immersed into 100 mL of each sample for five times and should be washed with deionized water to reach steady potential between the individual measurements. The mean potential was used to evaluate the content uniformity from the calibration graph.

Application to serum and urine

Adjust urine pH to 5 (using 0.1N hydrochloric acid) and pH of serum to 6(use phosphate buffer). Add hydrochloric acid to urine and phosphate buffer to serum dropwise until the suitable pH obtained. Transfer 5 mL previously adjusted urine or serum into small separatory funnels, and then separately add 5 mL, $1.0x10^{-3}$, $1.0x10^{-4}$ and 1.0×10^{-5} mol L^{-1} standard drug solution, followed by the addition of 20 mL toluene for urine or 20 mL diethyl ether for serum. Shake each funnel for 5 min, and transfer aqueous layer to centrifuge tube. Centrifuge for 2 min at 1500 rpm, transfer each solution to a 50 mL volumetric flask, and dilute to volume with deionized water. Apply above procedure as described under electrode calibration [21].

RESULTS AND DISCUSSION

Optimization of membrane composition

In the previous experimental investigations [22], it is obvious that both kind of plasticizer selected and the membrane composition used can influence the response performances (such as the sensitivity, linear concentration range, the detection limit, the response time etc.) of PVC membrane sensors, if other properties of the sensor, e.g. selectivity or pH response, are omitted. In this study four membrane compositions were investigated, the results were summarized in Table 1. The results showed that the electrode(s) made by membrane of type (d) with 9.6 w% clomipramine-reineckate ion pair, 48.0 w% PVC and 42.4 w% plasticizer DOP exhibits the best performance characteristics (slope 58.9±0.2, 59.33±0.1 and 56.07±0.5 mV $decade^{-1}$ at 25ºC for electrode I, II and III respectively, over clomipramine concentration range from$1.0x10^{-2}$-$5.0x10^{-6}$, $1.0x10^{-2}$-$1.0x10^{-6}$ mol L^{-1} and $1.0x10^{-2}$-$1.0x10^{-5}$ mol L^{-1}, for electrodes I, II and III, respectively.

Nature and response characteristics of sensors

Clomipramine reacts with ammonium reineckate to form a stable clomipramine-reineckate ion-pair complex which is water insoluble but readily soluble in an organic solvent such as tetrahydrofuran. The complex was prepared and tested as active material with di-octylphthalate DOP as a solvent mediator in a poly (vinyl chloride) membrane response for clomipramine. The critical response characteristics of plastic membrane, coated wire, and coated graphite rod-electrodes were determined and results are summarized in Table 2. The electrode(s) exhibits a Nernstain response over the concentration range from $1.0x10^{-2}$-$5.0x10^{-6}$, $1.0x10^{-2}$-$1.0x10^{-6}$ mol L^{-1} and $1.0x10^{-2}$-$1.0x10^{-5}$ mol L^{-1} clomipramine for electrode I, II and III, respectively with

a cationic slope of 58.9±0.2, 59.33±0.1 and 56.07±0.5 mV decade^{-1} change in concentration for electrodes I, II and III, respectively as in Figure 2. The choice of membrane solvent to achieve the required selectivity is based on its electric permittivity and its immiscibility with aqueous phase, high viscosity, low solubility of the matrix in the membrane and ability to dissolve ion-pair complex. The response time of the electrode(s) was tested for $1.0x10^{-1}$-$1.0x10^{-6}$ mol L^{-1} clomipramine solutions. The sequence of measurements was from low to high concentrations. The electrode(s) exhibits a fast dynamic response of ≤ 10, for electrode I and II respectively, ≤ 25 for electrode III. The electrode(s) used for a period of 40, 48 and 35 days for electrodes I, II and III respectively, without significant change in the electrode(s) parameters.

Effect of plasticizer

In this study three plasticizers. DOP, DBP and DNP, were used to examine the optimization of the membrane with plasticizer entailed the use of plasticizer content ratio, 42.4, 47.5 and 46.0 w %, and the use of PVC contents of 48.0, 47.5 and 47.0 w%. The electroactive compound (clomipramine–reineckate) contents were of 9.6, 7.0, 5.0 and 9.6 w%. The results obtained showed that the response performances of the prepared were rather different depending on the use of plasticizer, the proportion of the plasticizer toward PVC and of the electroactive compound. The typical potential responses of the electrodes constructed with three plasticizers were given in Figure 3. As shown in Figure 3, the DOP-PVC electrodes were superior to DBP- and DNP-PVC electrodes in both the response slope and linear concentration range. So DOP was selected as the plasticizer of the membranes. The best membrane composition of the DOP-PVC electrode(s) was 48.0 w % PVC, 42.4 w % DOP and 9.6 w % ion-pair.

Effect of soaking

The performance characteristics of clomipramine-reineckate electrode(s) was studied as a function of soaking time. For this purpose the electrode(s) was soaked in $1.0x10^{-3}$ mol L^{-1} solution of clomipramine hydrochloride and the calibration graphs were plotted after 3, 2, and 6 h. the optimum soaking time was found to be 3, 2 and 6 h at which the slope of the calibration curve was 58.90±0.2, 59.33±0.1 and 56.07±0.5 mV decade^{-1}, at 25 ºC for electrodes I, II and III, respectively. The influence of prolonged soaking on the lifetime of clomipramine-reineckate electrode(s) was followed by constructing calibration plots. The electrode(s) was soaked continuously on 1.0×10^{-3} mol L^{-1} solution of clomipramine hydrochloride for 7, 12, 20, 25, 35 and 40 days. The calibration plot slopes decreased slightly to 55.58, 56.95 and 54.53 mV decade^{-1} after 20 days for electrode I, II and III, respectively and continued to decrease reaching 52.09, 54.40 and 48.86 mV decade^{-1} after 35 days. The slope of the electrode(s) was dropped to 48.37, 52.21 and 42.38 mV decade^{-1} after 40 days for electrode I, II and III, respectively. Figure 4, shows the effect of prolonged soaking time and the life span of the clomipramine-reineckate plastic membrane electrode.

Regeneration of the electrode

The above discussion reveals that soaking of the electrode(s) in the drug solution for a long time has a negative effect on the response of the membrane. The same effect appears after working with the electrode(s) for a long time. The regeneration of the electrode(s) was tried simply by reformation of the ion-exchange on the external gel layer of membrane [23]. The regeneration of the clomipramine membrane was successfully achieved by soaking the exhausted electrode(s) for 24 h in a solution that was 1.0×10^{-2} mol L^{-1} ammonium reineckate, followed by soaking for 3 h in $1.0x10^{-2}$ mol L^{-1} clomipramine hydrochloride solution. Figure 5-7, show the calibration graphs for an exhausted electrode(s) (slopes 48.37, 52.21 and 42.38 mV decade^{-1}) for electrode I, II and III respectively, and for the same electrode(s) after regeneration (slopes 54.24, 55.20 and 52.19 mV decade^{-1}) for electrode I, II and III respectively. It was found that the lifespan of the regenerated electrode(s) is limited to 3-4 h due to the ease of leaching of the lipophilic salts from the gel layer at the electrode(s) surface compared with those that are attached homogeneously to the PVC network through the solvent mediator.

Effect of pH

The effect of pH of the clomipramine hydrochloride solutions ($1.0x10^{-3}$ mol L^{-1} clomipramine) on the electrode(s) potential was investigated. The solutions were acidified by the addition of very small volumes of hydrochloric acid 0.1N then the pH value was increased gradually using sodium hydroxide 0.1N for each pH value, the potential was recorded and thus the potential-pH curves for clomipramine concentrations were constructed as in Figure 8. As is obvious, within the pH range 3-8, the electrode(s) potential is practically independent of pH, and in this range the electrode can be safely used for clomipramine hydrochloride determination. The potential decrease at higher pH values is most probably attributed to the formation of the ionization of the hydroxyl group, leading to a decrease in the concentration of the clomipramine.

Selectivity of the electrode

Potentiometric selectivity coefficients were evaluated by the separate solution method. Table 3 showed that the proposed clomipramine-reineckate membrane electrode(s) is highly selective toward clomipramine. The electrode(s) showed no response to a number of potentially interfering ionic excipients usually used in the manufacturing of the pharmaceutical

preparations, such as starch and lactose. In the case of amino acids, the high selectivity is mainly attributed to the difference in polarity and lipophilic character of their molecules relative to clomipramine.

Quantification of clomipramine hydrochloride

Direct potentiometric determination of clomipramine hydrochloride using clomipramine-reineckate electrode(s) type I, II and III, was performed and calculated from the calibration curve. The direct potentiometric determination of clomipramine hydrochloride in pure form using the proposed electrodes gave average recovery % of 99.8±0.8, 99.5±0.5 and 99.4±0.7 for electrode I, II and III respectively.

Furthermore, the results obtained were encouraging so the proposed method was applied for the determination of clomipramine in its pharmaceutical preparations, the results compared with the official method [24], (potentiometric titration using 0.1 sodium hydroxide, for determination of clomipramine), and the results are listed in Table 4. The proposed method was successfully applied for the determination of clomipramine hydrochloride in biological fluids the results are listed in Table 5.

Method validation

The accuracy of the proposed method was investigated by the determination of clomipramine hydrochloride in spiked placebo samples prepared from serial concentrations of clomipramine reference standards.

The results summarized in Table 6, show that the proposed method is an accurate one for the determination of clomipramine hydrochloride in their pharmaceutical preparations without interfering from the coformulated adjuvants as indicated by the percentage recovery values.

The linearity, under the optimal experimental conditions, linear relationships exist between the electrode potential/mV and the logarithm of corresponding concentration of the investigated drug. The regression data, correlation coefficients (r) and other statistical parameter are previously listed in Table 2.

The precision of the proposed ISE method, measured as percentage relative standard deviation (%RSD) was tested by repeating the proposed method for determination of the investigated drug in its pharmaceutical preparations to nine replicates. The RSD% values for the repeated determinations were 0.194 %, 0.551% and 0.482% for determination of clomipramine hydrochloride in Supranil® capsule using electrode I, II and III, respectively and 0.820 %, 0.693 % and 0.535 % in Anafranil® Tablets using electrode I, II and III respectively. The above RSD% values are less than 2% indicating good precision.

The robustness of the proposed method was tested by investigating the capacity of the method to remain unaffected by a small but a deliberate variation in method parameters and provide an indication of its reliability during normal usage. While the ruggedness of the proposed method was investigating the degree of reproducibility at test results obtained by the analysis of the same samples under a variety of conditions such as different laboratories, analysts and instruments.

The results obtained by using another model of pH-meter (Orion 420 A) were compared with those obtained using model of pH-meter (Jenway 3040). The obtained results are close and also reveal validity of the method. The results previously listed in Table 2. The detection limit of the investigated drug 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 values were previously reported in Table 2 indicate that the proposed ISE method is sensitive for detection of very small concentrations of clomipramine hydrochloride.

Table 1. Optimization of membrane composition (w/w %)

Type of Sensor	m	PVC w%	DOP w%	Ion-Pair w %	Slope	RSD%	r*	Linear Conc. Range
Plastic Membrane Electrode	(a)	47.5	47.5	0.5	52.5	0.1	0.9999	1.0×10^{-2}-5.0×10^{-5}
	(b)	47.0	46.0	0.7	54.7	0.4	0.9997	1.0×10^{-2}-1.0×10^{-5}
	(c)	45.0	45.0	10.0	57.2	0.1	0.9998	1.0×10^{-2}-9.0×10^{-5}
	(d)	48.0	42.4	9.6	58.9	0.2	0.9997	1.0×10^{-2}-5.0×10^{-6}
Coated Wire Electrode	(a)	47.5	47.5	0.5	56.27	0.3	0.9996	1.0×10^{-2}-1.0×10^{-5}
	(b)	47.0	46.0	0.7	55.90	0.1	0.9998	1.0×10^{-2}-1.0×10^{-5}
	(c)	45.0	45.0	10.0	57.83	0.5	0.9993	1.0×10^{-2}-5.0×10^{-5}
	(d)	48.0	42.4	9.6	59.33	0.1	0.9999	1.0×10^{-2}-1.0×10^{-6}
Coated Graphite Electrode	(a)	47.5	47.5	0.5	47.70	0.2	0.9991	1.0×10^{-2}-1.0×10^{-4}
	(b)	47.0	46.0	0.7	52.50	1.1	0.9995	1.0×10^{-2}-5.0×10^{-4}
	(c)	45.0	45.0	10.0	53.80	1.2	0.9993	1.0×10^{-2}-9.0×10^{-4}
	(d)	48.0	42.4	9.6	56.07	0.1	0.9997	1.0×10^{-2}-1.0×10^{-5}

Table 2. Critical response characteristics of sensors

Parameter[a]	Clomipramine-RK plastic membrane electrode	Clomipramine-RK coated wire electrode	Clomipramine-RK coated graphite electrode
Slope (mV decade^{-1})	58.9±0.2	59.3±0.1	56.0±0.5
Intercept	376.79	563.70	426.01
Correlation coefficient r.	0.9997	0.9999	0.9997
Linear range (mol L^{-1})	$1.0x10^{-2}$-5.0×10^{-6}	1.0×10^{-2}-1.0×10^{-6}	1.0×10^{-2}-1.0×10^{-5}
Detection limit (mol L^{-1})	1.9×10^{-6}	4.8×10^{-7}	5.0×10^{-6}
Response time /(s)	≤10	≤10	≤25
Working pH range	4-8	4-8	4-8
Lifetime /day	40	40	35
Accuracy (%)	99.59	99.66	99.40
Standard deviation (%)	0.5	0.5	0.8
Repeatability (CV_w%)	0.6	0.7	0.9
Between day variability (CV_b %)	0.9	0.8	0.8
Robustness[b]	99.85±0.1	99.65±0.3	99.58±0.2
Ruggedness[c]	99.91±0.4	99.84±0.6	99.78±0.8

[a]Mean of six measurements, [b]A small variation in method parameters were as pH of buffer.
[c]Comparing the results by those obtained by different sensors assemblies using (Orion 420A)

Table 3. Selectivity coefficient and tolerance values for clomipramine hydrochloride sensors

	Clomipramine-RK plastic membrane electrode $K^{pot}_{clom^+Cl^-}$	Clomipramine-RK coated wire electrode $K^{pot}_{clom^+Cl^-}$	Clomipramine-RK coated graphite electrode $K^{pot}_{clom^+Cl^-}$
Na^+	1.9×10^{-3}	1.6×10^{-4}	1.4×10^{-3}
K^+	3.7×10^{-3}	3.9×10^{-4}	4.1×10^{-3}
Ca^{2+}	1.5×10^{-3}	1.7×10^{-3}	1.1×10^{-3}
Mg^{2+}	6.7×10^{-4}	5.8×10^{-4}	7.1×10^{-4}
L- Systin	3.6×10^{-4}	2.5×10^{-4}	4.5×10^{-4}
L- Leucin	2.1×10^{-4}	6.7×10^{-4}	5.2×10^{-4}
Starch	1.2×10^{-4}	1.9×10^{-4}	1.4×10^{-3}
Lactose	2.8×10^{-4}	1.5×10^{-3}	2.4×10^{-3}
Urea	1.8×10^{-4}	3.6×10^{-3}	4.1×10^{-3}
Gabapentin	5.5×10^{-3}	4.9×10^{-3}	6.3×10^{-3}
Chlopromazine HCl	1.4×10^{-1}	1.5×10^{-2}	1.2×10^{-2}
Nalbuphine HCl	2.4×10^{-4}	3.1×10^{-4}	2.9×10^{-4}
Paroxetine HCl	1.6×10^{-3}	2.4×10^{-3}	1.8×10^{-3}

Table 4. Determination of clomipramine hydrochloride in pure form and pharmaceutical formulations in comparison with official method

Types Of sensors	Statistical parameter	Official method	Direct Potentiometry	
			Calibration method	Standard addition method
Clomipramine-RK plastic membrane electrode	Pure sample Mean±SD	99.57±0.579	99.78±0.776	99.26±0.955
	SE	0.236	0.317	0.390
	RSD	0.582	0.778	0.962
	"t"		(0.531)(2.228)*	(0.680)(2.228)*
	F		(1.79)(5.05)*	(2.72)(5.05)*
	Supranil®25 mg/Cap Mean±SD	99.14±0.427	99.20±0.677	99.34±0.363
	SE	0.174	0.276	0.162
	RSD	0.431	0.682	0.365
	"t"		(0.184)(2.228)*	(0.840)(2.228)*
	F		(2.52)(5.05)*	(1.38)(5.19)*

Clomipramine-RK coated wire electrode	Pure sample Mean±SD SE RSD "t" F Supranil®25 mg/Cap Mean±SD SE RSD "t" F	99.08±0.613 0.250 0.619 98.99±0.666 0.272 0.673	99.50±0.475 0.180 0.477 (1.360)(2.201)* (1.66)(4.39)* 98.51±0.778 0.294 0.790 (1.198)(2.201)* (1.36)(4.39)*	99.30±0.446 0.182 0.449 (0.711)(2.228)* (1.88)(5.05)* 98.77±0.710 0.289 0.719 (0.554)(2.228)* (1.14)(5.05)*
Clomipramine-RK coated graphite electrode	Pure sample Mean±SD SE RSD "t" F Supranil®25 mg/Cap Mean±SD SE RSD "t" F	99.11±0.874 0.357 0.882 98.86±0.668 0.298 0.676	99.45±0.695 0.263 0.699 (0.767)(2.201)* (1.53)(4.39)* 98.09±0.616 0.251 0.628 (1.976)(2.262)* (1.18)(5.19)*	99.22±0.563 0.229 0.567 (0.259)(2.228)* (2.41)(5.05)* 97.96±0.912 0.372 0.931 (1.888)(2.262)* (1.87)(5.19)*

Table 5. Determination of clomipramine hydrochloride in human serum and urine

Types of sensors	Statistical parameter	Direct Potentiometry	
		Calibration method	Standard addition method
Clomipramine-RK plastic membrane electrode	Serum sample Mean±SD n Variance %SE %RSD Urine sample Mean±SD N Variance %SE%RSD	98.74±0.841 5 0.707 0.376 0.852 99.04±0.424 5 0.179 0.189 0.428	98.99±0.587 5 0.345 0.263 0.593 98.94±0.917 5 0.841 0.410 0.927
Clomipramine-RK coated wire electrode	Serum sample Mean±SD n Variance %SE %RSD Urine sample Mean±SD n Variance %SE %RSD	98.70±0.753 6 0.567 0.307 0.763 98.51±0.445 6 0.198 0.182 0.452	99.20±0.614 6 0.376 0.251 0.619 99.03±0.676 6 0.457 0.276 0.683
	Serum sample Mean±SD n	98.76±0.833 5	98.41±0.464 5

Clomipramine-RK coated graphite electrode	Variance	0.694	0.215
	%SE	0.373	0.208
	%RSD	0.843	0.471
	Urine sample		
	Mean±SD	98.44±0.713	98.32±0.896
	n	5	5
	Variance	0.508	0.803
	%SE	0.319	0.401
	%RSD	0.724	0.911

Table 6. Determination of Clomipramine hydrochloride in clomipramine-spiked placebo samples using clomipramine-reineckate sensors

Types of electrodes	Sample of capsules placebo			Sample of tablets placebo		
	Added (mol L^{-1})	Found -log conc. (mol L^{-1})	Recovery %	Added (mol L^{-1})	Found -log conc. (mol L^{-1})	**Recovery %**
Plastic membrane electrode	$1x10^{-6}$	5.99	99.83	$1x10^{-6}$	5.96	**99.33**
	$3x10^{-6}$	5.49	99.82	$3x10^{-6}$	5.43	**98.73**
	$5x10^{-6}$	5.28	99.60	$5x10^{-6}$	5.31	**100.17**
	$1x10^{-5}$	4.97	99.40	$1x10^{-5}$	4.99	**99.80**
	$3x10^{-5}$	4.51	100.22	$3x10^{-5}$	4.42	**98.22**
	$5x10^{-5}$	4.26	99.05	$5x10^{-5}$	4.32	**100.44**
	$1x10^{-4}$	3.98	99.50	$1x10^{-4}$	3.95	**98.75**
	$3x10^{-4}$	3.45	98.57	$3x10^{-4}$	3.49	**99.71**
	$5x10^{-4}$	3.31	100.30	$5x10^{-4}$	3.27	**99.06**
Statistical parameters	N	9		N	**9**	
	Mean	99.59		Mean	**99.36**	
	SD	0.546		SD	**0.733**	
	RSD	0.548		RSD	**0.738**	
Coated wire electrode	$1x10^{-6}$	5.96	99.33	$1x10^{-6}$	6.01	**100.17**
	$3x10^{-6}$	5.41	98.36	$3x10^{-6}$	5.46	**99.27**
	$5x10^{-6}$	5.29	99.25	$5x10^{-6}$	5.24	**98.85**
	$9x10^{-9}$	4.03	99.61	$9x10^{-9}$	5.02	**99.49**
	$1x10^{-5}$	4.99	99.80	$1x10^{-5}$	5.00	**100.00**
	$3x10^{-5}$	4.48	99.56	$3x10^{-5}$	4.46	**99.11**
	$5x10^{-5}$	4.27	99.28	$5x10^{-5}$	4.31	**100.21**
	$9x10^{-5}$	4.04	99.86	$9x10^{-5}$	4.05	**100.11**
	$1x10^{-4}$	4.01	100.25	$1x10^{-4}$	3.99	**99.75**
Statistical parameters	N	9		N	**9**	
	Mean	99.48		Mean	**99.66**	
	SD	0.528		SD	**0.504**	
	RSD	0.531		RSD	**0.506**	
Coated graphite electrode	$1x10^{-5}$	4.95	99.00	$1x10^{-5}$	4.97	**99.40**
	$3x10^{-5}$	4.47	99.33	$3x10^{-5}$	4.50	**100.00**
	$5x10^{-5}$	4.26	99.05	$5x10^{-5}$	4.26	**99.05**
	$9x10^{-5}$	4.05	100.11	$9x10^{-5}$	4.00	**98.87**
	$1x10^{-4}$	3.99	99.75	$1x10^{-4}$	3.96	**99.00**
	$3x10^{-4}$	3.49	99.71	$3x10^{-4}$	3.42	**97.71**
	$5x10^{-4}$	3.22	97.55	$5x10^{-4}$	3.29	**99.67**
	$9x10^{-4}$	3.04	99.81	$9x10^{-4}$	3.03	**99.48**
	$1x10^{-3}$	3.01	100.33	$1x10^{-3}$	2.99	**99.67**
Statistical parameters	**N**	**9**		**N**	**9**	
	Mean	**99.40**		**Mean**	**99.21**	
	SD	**0.827**		**SD**	**0.670**	
	RSD	**0.832**		**RSD**	**0.675**	

Figure 1. Chemical structure of clomipramine hydrochloride

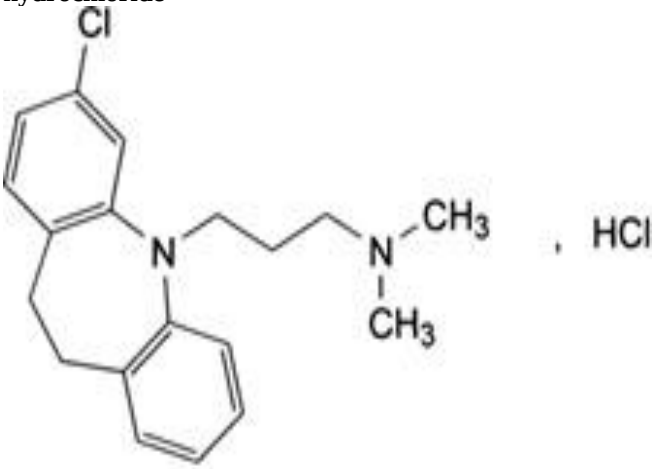


Figure 2. Typical calibration graph of clomipramine sensors: (▼) Clomipramine- plastic membrane ,(●) Clomipramine-coated wire, (□) Clomipramine-coated graphite electrode

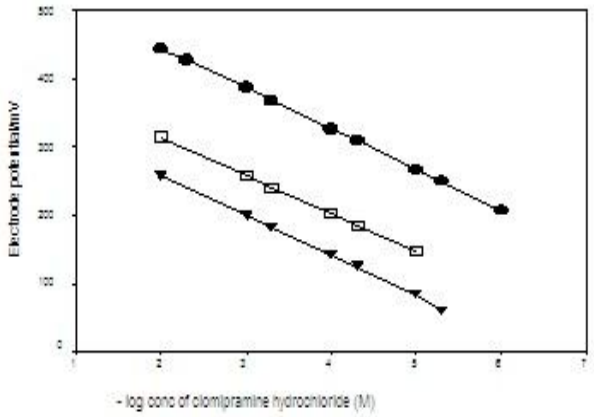


Figure 3. Optimization of plasticizers: DOP (▼) (PVC membrane composition DOP 42.4 wt%, PVC 48.0 wt%, ion pair, 9.6 wt %), DBP (□) (PVC membrane composition: DBP 47.5 wt%, PVC 47.5 wt%, ion-pair, 5.0 wt %), DNP (●) (PVC membrane Composition: (DNP 46.0 wt %, PVC 47.0 wt%, ion-pair, 7.0 wt %)

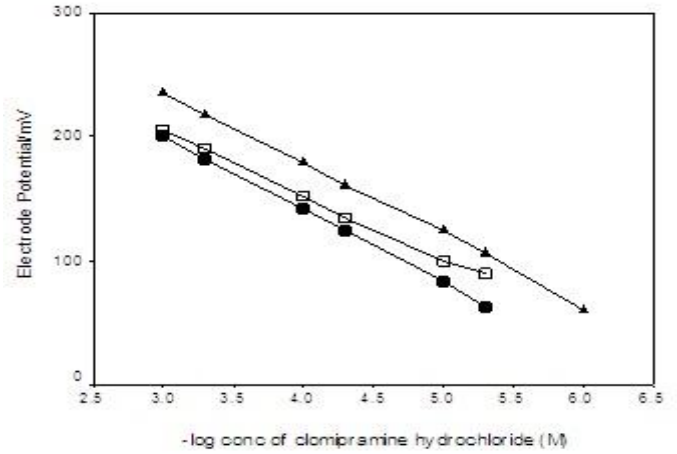


Figure 4. Calibration graphs obtained at 25±1°C after soaking the clomipramine-reineckate plastic membrane electrode for (●)7 days, (□) 12 days, (■) 20 days, (▼) 25 days, (+) 35 days, (○) 40 days

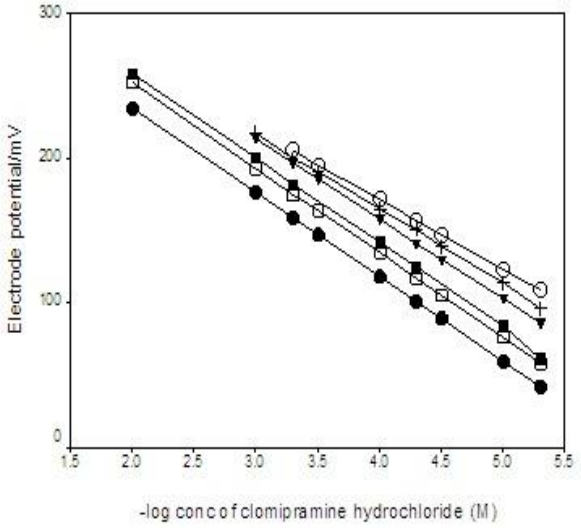


Figure 5. Regeneration of clomipramine-reineckate plastic membrane sensor (●) exhausted electrode (■) regenerated electrode

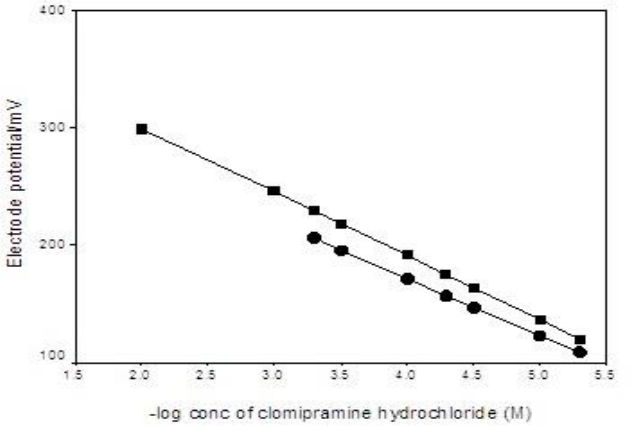


Figure 6. Regeneration of clomipramine-reineckate coated wire sensor (●) exhausted electrode (■) regenerated electrode

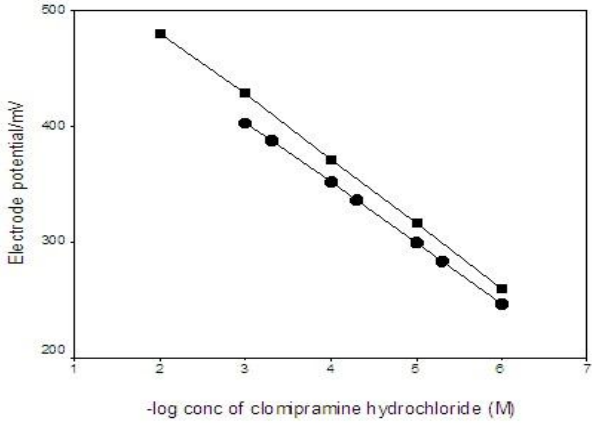


Figure 7. Regeneration of clomipramine-reineckate coated graphite sensor (●) exhausted electrode (■) regenerated electrode

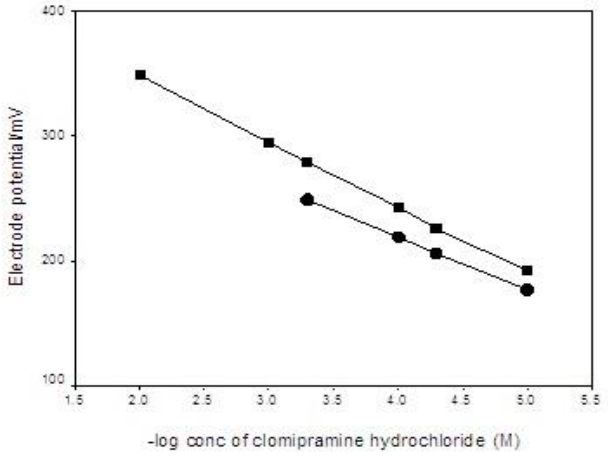
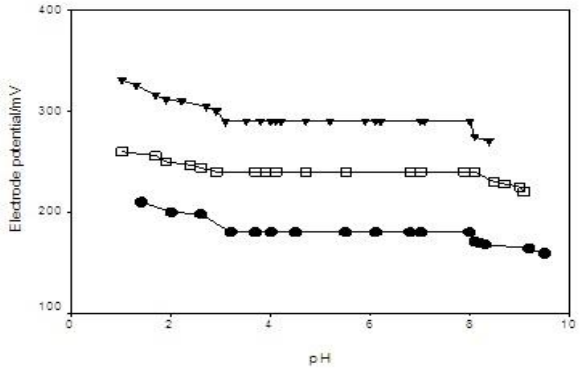


Figure 8. Effect of pH on potential /mV of Clomipramine-reineckate sensors using 1x10^{-3}M (●) Electrode potential/mV for plastic membrane electrode, (□) Electrode potential/mV for coated wire electrode, (▼) Electrode potential/mV for coated graphite lectrode



Electrode response in pharmaceuticals and biological fluids

The uses of clomipramine hydrochloride drug in various clinical fields has been necessitated an accurate and rapid, quantitative analysis in various matrices (dosage forms and biological fluids). This work proposed a fast, simple, easy, sensitive and straightforward potentiometric method to determine clomipramine in dosage forms without the need for prior separation and preconcentration or derivatization procedures.

The potential of the clomipramine-reineckate sensors showed no significant difference of response time between aqueous solution of pure drug and its solutions from pharmaceutical preparations and biological fluids. The proposed method described good accuracy and precise for the quality control tests, the content uniformity assay showed that the (RSD <1%), with mean standard deviation 99.42±0.7, 99.69±0.7 and 99.38±0.4 for electrodes I, II and III respectively.

CONCLUSION

The proposed method has some important advantages: the electrode(s) proved to be successful, providing a rapid, simple and low cost potentiometric method for the determination of clomipramine hydrochloride in pure form, pharmaceutical preparations and biological fluids. The present electrode(s) is easily and simply regenerated and ensures a good accuracy in term of content uniformity assay.

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