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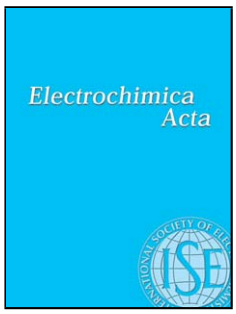

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Drug Selective Poly (Vinyl chloride)-based Sensor of Desipramine Hydrochloride

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Abstract

The novel ion-pair ($[TPB]^-$ $[DH]^+$) of the quaternary ammonium drug desipramine hydrochloride, 3-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)-N-methylpropan-1-amine, has been synthesized and incorporated into a poly(vinyl chloride)-based membrane sensor for the quantification of desipramine hydrochloride in different pharmaceutical preparations. The influence of the membrane composition on the potentiometric responses of the membrane sensor has been found to substantially improve the performance characteristics. The best performance was reported with membranes having the composition (in mg) of ($[TPB]^-$ $[DH]^+$) (5): PVC (150): *o*-NPOE (150). The proposed sensor (sensor no. 4) exhibits a nernstian response in the concentration range of 2.2×10^{-6} – 1.0×10^{-2} M with a detection limit of 1.2×10^{-6} M. The membrane sensor performs satisfactorily over the pH range of 2.8 – 7.4 with a fast response time of 12 seconds. The sensor no. 4 can tolerate a non-aqueous content of up to 20% and can be utilized for the determination of drug concentration in pharmaceutical preparation (tablets) and in body fluids such as urine and blood samples. The results were comparatively evaluated with Liquid Chromatography (LC). It was observed that the concentration of drug was greater in the blood sample than in the urine sample, as most of the drug is metabolized in the liver before discharge to urine.

Keywords: Quaternary ammonium drug, Desipramine Hydrochloride, Poly(vinyl chloride) membrane sensor,

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1. Introduction

Desipramine hydrochloride, 3-(5,6-dihydrobenzo[b][1] benzazepin-11-yl)-N-methylpropan-1-amine, a tertiary amine tricyclic antidepressant, is structurally related to both the skeletal muscle relaxant cyclobenzaprine and the thioxanthene antipsychotics such as thiothixene. Desipramine is used to treat depression, pain of neuropathic origin, attention deficit hyperactivity disorder, functional enuresis in children, panic and phobic disorder and to manage some eating disorders [1-3]. Desipramine inhibits the re-uptake of noradrenalin at the noradrenergic nerve endings and the re-uptake of serotonin (5-hydroxytryptamine) at the serotoninergic nerve endings in the central nervous system. These two effects are considered to be the likely basis of the antidepressant effect of desipramine. The drug also has a strong anticholinergic effect and serves as an antagonist on a1 and H1 receptors. Therefore, its quantification is necessary in different biological samples and bulk formulations, as well as in different finished product dosages forms such as tablets, capsules, injections, etc. Several analytical methods have been reported as generic methods for most antidepressants or exclusively for the quantitative determination of desipramine hydrochloride (DH) and its metabolites in biological samples using capillary electrophoresis, Gas Chromatography, Liquid Chromatography, UV and mass spec detection [4–15]. All of these methods require time-consuming and costly sample pretreatment.

Thus, there is critical need for the development of selective, portable, inexpensive diagnostic tools for the determination of this analyte. Analytical methods based on potentiometric detection with ion-selective electrodes (ISEs) can be considered as an advantageous alternative because they are eco-friendly techniques, provide easy construction and manipulation, present good selectivity in a wide concentration range, a relatively low detection limit, show fast response and perform non-destructive analysis. This has led to increasing interest by our research group in the development and application of an ion-selective electrode using various cyclic and acyclic neutrals and ion-pairs for the determination of metal [16-21] and non-metal ions [22-23], organic molecules [24] and some selective drugs [25-32]. With this intent, we synthesized an NaTPB-based ion pair of desipramine hydrochloride $[TPB]^-$ $[DH]^+$ and used it as a carrier molecule in a PVC-based membrane sensor for the determination of desipramine hydrochloride in biological samples and in different drug formulations.

2. Experimental

2.1. Reagents and materials

High molecular weight polyvinyl chloride (PVC) and desipramine hydrochloride were obtained from Aldrich (Wisconsin, USA); *o*-nitrophenyl octyl ether (*o*-NPOE) and potassium tetrakis(4-chlorophenyl borate) (KTpClPB), from Fluka (Ronkonkoma, NY); tri-*n*-butylphosphate (TBP), from BDH (Poole, England); chloronapthalene (CN), dibutylphthalate (DBP), sodium tetraphenylborate (NaTPB) and dibutyl(butyl) phosphonate (DBBP), from Mobile (Alabama, USA); drug standard, from CDL (Central Drug Laboratory, Calcutta, India).

2.2 Preparation of ion-pair complex

The ion pair $[TPB]^-[DH]^+$ has been synthesized by separately preparing equimolar solutions (1.0×10^{-3} M) of NaTPB and desipramine hydrochloride (DH), each in 50 mL of ethanol, in round-bottom flasks, dissolved by moderate heating with a magnetic stirrer. Subsequently, small aliquots of dissolved DH filled by burette were added to the round-bottom flask of NaTPB with a magnetic stirrer at 78°C. When all of the content was added, the mixture was retained with magnetic stirring for 30 minutes for complete mixing, followed by refluxing for 2 hours over $CaCl_2$ to remove the moisture, and storing overnight. In the morning, light yellowish-white precipitate was observed. The precipitate was filtered, washed with small aliquots of ethanol and recrystallized with ethanol.

$[TPB]^-$ $[DH]^+$. Yield: 68%; color: yellowish-white; U.V-vis ($\lambda_{max/nm}$) (0.005% w/v methanol): 251, 255. Elemental analysis (by %) was observed: C = 86.05, B = 1.72, N = 4.71, H = 7.06, and calculated % was C = 86.09, B = 1.80, N = 4.76, H = 7.16. The observed elemental analysis is consistent with the theoretical data obtained on the basis of the structure as given in Figure 1.

2.3. Stoichiometry of $[TPB]^-$ $[DH]^+$

The discussion of stoichiometry of ion-pair $[TPB]^-$ $[DH]^+$ was necessary to prove the association of ionic species performed using Job's method. The concentrations of desipramine hydrochloride (DH) and NaTPB were taken to be constant (1.5×10^{-3} M). Nine methanolic solutions were prepared containing desipramine hydrochloride and NaTPB in various molar ratios so that the final volume always amounted to 10 mL after the addition of phosphate buffer (0.04 M) pH 5.5.

The extraction was performed using 10 mL of chloroform, and the absorbance was measured at 251 nm. The plot reached a maximum value at mole fraction X_{max} = 0.5 (Figure 2), which indicates the formation of 1:1 ion-pair association.

2.4. Electrode fabrication

The PVC-based membranes were prepared by dissolving appropriate amounts of ion pair $[TPB]^-$ $[DH]^+$, solvent mediators dioctyl phthalate (DOP), tri-*n*-butylphosphate (TBP), 1-chloronaphthalene (CN) and *o*-nitrophenyl octyl ether (*o*-NPOE) and appropriate amounts of PVC in THF (5-10 mL). After complete dissolution of all components and thorough mixing, the resulting mixture was poured into polyacrylate rings placed on a smooth glass plate. THF was allowed to evaporate for about 24 h at 25 ^oC. To obtain membranes with reproducible characteristics, the viscosity of the solution and solvent evaporation were carefully controlled; otherwise, morphology and thickness of the membranes have shown drastic variations that ultimately affected the sensor response. The transparent membranes of 0.4 mm thickness were carefully removed from the glass plate. A 5 mm diameter piece was cut out and glued to one end of a Pyrex glass tube. The membranes thus prepared were equilibrated for 2-3 days in a standard drug solution of 1.0×10^{-2} M. Membranes of different compositions were prepared and investigated. Ones that gave reproducible results and best performance characteristics were selected for detailed studies. The optimum composition of membranes for best performance is given in Table 1. It was reported that sensor no. 4 shows the best performance in terms of detection limit (1.2×10^{-6} M), working range ($2.2 \times 10^{-6} – 1.0 \times 10^{-2}$ M), slope (59.2 mV/decade of activity) and response time (12 s).

2.5. Conditioning of membranes and potential measurements

The membrane electrode bodies containing an inner solution of 1.0×10^{-1} M prepared as above were equilibrated for 2-3 days in a standard drug solution of 1.0×10^{-2} M using phosphate buffer (0.04 M) pH 5.5 prior to potential measurements. The potential measurements were carried out at 25±1^oC using a saturated calomel electrode (SCE) as the reference electrode with the following cell assembly: $Hg/Hg_2Cl_2|KCl$ (satd.)|0.1M DH||PVC membrane||test solution|$Hg/Hg_2Cl_2|KCl$ (satd.)

3. Results and Discussion

3.1. Calibration of electrode

Calibration of the sensor's detection parameters such as the detection limit and the working range was performed by response to the standard drug solution. The membrane holder assembly was immersed in 1.0×10^{-2} M standard drug solution, the inner compartment filled with 1.0×10^{-1} M solution for 2 days, and potentiometric responses were reported for $1.0 \times 10^{-7} - 1.0 \times 10^{-2}$ M standard drug dilutions in phosphate buffer (0.04 M) pH = 5.5 for comparative analysis of real samples. The results are plotted in Figure 3. For the standard drug solution, sensor no. 4 shows a detection limit of 1.0×10^{-6} M and a working range of $2.0 \times 10^{-6} - 1.0 \times 10^{-2}$ M.

3.2. Selectivity coefficient

The selectivity of the membrane sensor is one of the most important performance parameters that determine the utility of the sensor. Thus, selectivity studies were carried out only for sensor no. 4, which exhibited the best performance characteristics in terms of working concentration range, slope, response time and lifetime. The selectivity coefficients ($K_{DH^+,\ B}^{Pot.}$) were determined by the modified form of fixed interference method (FIM), as represented by equation no. 1 suggested by Saez de Viteri and Diamond [33], and are given in Table 2. Thus, sensor no. 4 is highly selective to DH^+ ions above all of the interfering ions and organic molecules studied and listed in Table 2. Thus, the selectivity coefficient indicates that it is possible to determine DH^+ concentration. It is important to note that the concentration of interfering ions that the sensor can tolerate for levels of DH^+ below or slightly above that of the interfering ion depends on the numerical value of the selectivity coefficient. Smaller values of the selectivity coefficient indicate higher tolerance for concentration of interfering ion(s) by the sensor. To have a practical idea of the concentration level that can be tolerated, mixed run studies were carried out in the presence of different concentrations of Imipramine HCl (IH), which showed higher values of selectivity coefficient for proposed sensor no. 4. In this measurement, the potential of the sensor was determined as a function of DH^+ concentration at various IH^+ concentrations, and the results are shown in Figure 4. The linear portion of the plot is the reduced working concentration range that can tolerate the IH^+ concentration causing deviation. Cell potential varies with activity of DH^+ at different concentration levels of IH^+ ions (sensor no. 4). 1.0×10^{-5} M is tolerated over the entire working

concentration range (2.2×10^{-6} to 1.0×10^{-2} M), as it causes no deviation. However, IH^+ concentrations of 1.0×10^{-4}, 1.0×10^{-3} and 1.0×10^{-2} M cause deviation in the pure solution plot of DH^+. Thus, these results show that the sensor can be used to determine DH^+ even in the presence of IH^+ at concentrations several times that of DH^+. The performance of the sensor with regard to the interference effect of other ions is better still because their selectivity coefficient values are smaller than that for IH^+.

$$K_{DH^+,\ B}^{Pot} = \frac{aDH^+}{(aB)^{zA/zB}} \qquad (1)$$

3.3. Effect of pH

The effect of pH on the performance of the most responsive membrane sensor, no. 4, was studied over the pH range of 2 to 8 using two concentrations, 1.0×10^{-4} M and 1.0×10^{-3} M of DH^+. The pH was adjusted by using dilute nitric acid and sodium hydroxide. The potential of the proposed sensor was determined as a function of pH, and the results are shown in Figure 5. The potential remains constant over the pH range of 2.8-7.4, which may be taken as the working pH range of the sensor assembly.

3.4. Non-aqueous effect

The performance of sensor no. 4 was further assessed in partial non-aqueous media, i.e., methanol-water, ethanol-water and acetonitrile-water mixtures. The results are compiled in Table 3 and show that no significant change occurs in the slope and working concentration of the sensor at up to 20% non-aqueous content. However, above 20% non-aqueous content, the working concentration of the sensor is significantly reduced; thus, the sensor can only be utilized in mixtures of up to 20% non-aqueous content.

3.5. Effect of plasticizers

It is well known that the sensitivity and selectivity of a cation-selective sensor strongly depend on the membrane composition and the nature of the plasticizer used [34,35]. The effect of

plasticizer on DH^+ selective membrane sensor no. 4 is shown in Table 4. It is clear from the corresponding table that *o*-NPOE is a more effective plasticizer than others in preparing the DH^+ selective membrane sensor. It is noteworthy that the lipophilicity of plasticizer influences both the dielectric constant of the polymeric membranes and the mobility of the ionophore and its metal complex [36,37]. This indicates that *o*-NPOE plasticizes the membrane, dissolves the ion association complexes and adjusts both the permittivity and the ion exchanger site mobility to give highest possible selectivity and sensitivity.**4. Analytical Application**

4.1 Application to pharmaceutical preparations

The proposed sensor number 4 has been applied for analysis of a commercial tablet of desipramine hydrochloride (50 mg) by using the standard addition method [38]. In the standard addition method, known small increments of a 1.0×10^{-2} M standard drug solution were added to 50.0 mL aliquots of various concentrations (1.0×10^{-6} – 1.0×10^{-2} M) of pharmaceutical preparations (tablet). The changes in potential were recorded for each increment and were used to calculate the concentration of the drug sample solution using the following equation:

$$C_x = C_s \left(\frac{V_s}{V_x + V_s}\right)\left(10^{n\left(\frac{\Delta E}{S}\right)} - \frac{V_x}{V_x + V_s}\right)^{-1} \qquad (2)$$

where C_x and V_x are the concentration and volume of the unknown sample, respectively, C_s and V_s are the concentration and volume of the standard, respectively, S is the slope of the calibration graph, and ΔE is the change in potential due to the addition of standards. The results are summarized in Table 5. During analysis, it was observed that sensor no. 4 work well in pharmaceutical preparation measurements.

4.2 Determination of desipramine hydrochloride in blood and urine samples

The proposed sensor number 4 has been applied for the determination of desipramine hydrochloride in blood and urine samples, and results were comparatively evaluated with LC. Seven volunteers (depression patients) selected for the study had taken 50 mg doses of desipramine HCl drug, and their blood and urine samples were collected at different time intervals. The complete arrangement was done under the supervision of doctors in a nearby city

hospital. The samples of urine and blood were separately centrifuged at 8000 rpm to remove the blood cells and other dead cells. Finally, 1 mL of each sample (urine & blood) was diluted using phosphate buffer (pH 6.5, 0.03 M) to 10 mL. The proposed sensor (no. 4) was directly used to measure the potential and to evaluate the drug concentration present in both samples (blood & urine) by using a calibration graph (Graph 3). The chromatographic separation was optimized in the YMC Pack Pro C_{18} column (150 × 4.6 mm and 3.0 µm as particle size). The gradient LC method uses water: trifluoroacetic acid in the ratio of 100 : 0.05 (v/v) as mobile phase A and acetonitrile: trifluoroacetic acid in the ratio of 100 : 0.08 (v/v) as mobile phase B. The flow rate of the mobile phase was 1.0 mL min^{-1}. The LC gradient program was set as (time/% mobile phase B): 0/15, 30/70 and 45/70 with a post-run time of 5 min. The column temperature was maintained at 40 ^{o}C, and the detection was set at a wavelength of 215 nm. The column loading was optimized as 6 µg of desipramine hydrochloride in 10 µL injection volume. A mixture of water and acetonitrile was used as a diluent in a 1:1 ratio. The results are presented in Table 6. The values obtained from the sensor are slightly higher in comparison to LC, which may be accounted for by the ISE uncertainty, since the Nernst relation is a log-linear one, enabling even small errors in the slope of a calibration curve to translate to relatively large absolute errors. However, it may be concluded that the proposed sensor is quite sensitive and can be used for the determination of drug concentrations in biological fluids. The estimated drug concentration is higher in blood samples in comparison to urine samples, as some part of the drug is metabolized, and excess drug is excreted in urine. As the active drug concentration is many times higher than that reported in Table 6, our proposed work has many applications to measure the drug concentration from the lowest level to a higher level, the latter also being a measure of toxicity.

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Figure captions

1. Structure of ion-pair $[TPB]^-$ $[DH]^+$

2. Stoichiometry of ion-pair $[TPB]^-$ $[DH]^+$, $X_{max} = 0.5$, phosphate buffer (0.04 M).

3. Calibration curve in response to the standard drug solution at pH 5.5 (phosphate buffer, 0.04 M), Detection limit 1.0×10^{-6} M, working range $2.0 \times 10^{-6} – 1.0 \times 10^{-2}$ M.

4. Interference study of Imipramine hydrochloride (IH^+) in detection of desipramine hydrochloride (DH^+) by sensor no. 4.

5. Effect of pH on the response of proposed sensor no. 4. (pH range 2.8-7.4).

Table 1. Optimization of membranes by varying the composition of ingredients.

Sensor no.	Composition of membranes (w/w), mg						Working concentration range (M)	Detection limit (M) ± *SD	Slope mV/decade of activity ± *SD	Response Time (s)
	$[TPB]^-$ $[DH]^+$	PVC	DOP	CN	*o*-NPOE	DBBP				
1	5	150	-	-	-	-	$2.1 \times 10^{-4} - 1.0 \times 10^{-2}$	$1.8 \times 10^{-4} \pm 0.04$	42.5 ± 0.05	23
2	5	150	150	-	-	-	$3.3 \times 10^{-5} - 1.0 \times 10^{-2}$	$2.8 \times 10^{-5} \pm 0.06$	55.6 ± 0.04	17
3	5	150	-	150	-	-	$1.3 \times 10^{-5} - 1.0 \times 10^{-2}$	$1.0 \times 10^{-5} \pm 0.05$	57.5 ± 0.06	14
4	5	150	-	-	150	-	$2.2 \times 10^{-6} - 1.0 \times 10^{-2}$	$1.2 \times 10^{-6} \pm 0.03$	59.2 ± 0.06	12
5	5	150	-	-	-	150	$6.3 \times 10^{-5} - 1.0 \times 10^{-2}$	$5.9 \times 10^{-5} \pm 0.06$	54.4 ± 0.03	18

*SD, Standard deviation for three consecutive measurements.

Table 2. Selectivity coefficient ($K_{DH^+,X}^{Pot.}$) values reported against the most responsive membrane sensor (Sensor no. 4).

Interfering ion (X)	Selectivity coefficient ($K_{DH^+,X}^{Pot.}$)
CH_3COO^-	1.3×10^{-4}
SCN^-	1.2×10^{-3}
*Neostigmine(+)	2.3×10^{-2}
*Propantheline(+)	2.1×10^{-2}
*Lofepramine (+)	1.8×10^{-2}
NH_4^+	2.3×10^{-3}
*Imipramine(+)	3.5×10^{-1}
Na^+	1.4×10^{-4}
Ca^{2+}	2.1×10^{-3}
Ba^{2+}	2.5×10^{-3}
**2-hydroxydesipramine(+)	4.5×10^{-2}

*Quaternary ammonium drug salts

**Metabolite of desipramine hydrochloride

Table 3. Non-aqueous effect on the performance of the most responsive membrane sensor

Non–aqueous content (%v/v)	Working concentration range (M)	Slope (± 0.2 mV decade^{-1} of activity)
0	2.2×10^{-6} - 1.0×10^{-2}	59.2
Methanol		
10	2.2×10^{-6} - 1.0×10^{-2}	59.2
15	2.2×10^{-6} - 1.0×10^{-2}	59.2
20	2.3×10^{-6} - 1.0×10^{-2}	59.1
25	4.8×10^{-6} - 1.0×10^{-2}	58.1
30	8.4×10^{-5} - 1.0×10^{-2}	57.5
Ethanol		
10	2.2×10^{-6} - 1.0×10^{-2}	59.2
15	2.1×10^{-6} - 1.0×10^{-2}	59.2
20	2.2×10^{-6} - 1.0×10^{-2}	59.3
25	5.2×10^{-6} - 1.0×10^{-2}	58.6
30	8.2×10^{-5} - 1.0×10^{-2}	57.8
Acetonitrile		
10	2.2×10^{-6} - 1.0×10^{-2}	59.2
15	2.2×10^{-6} - 1.0×10^{-2}	59.2
20	2.1×10^{-6} - 1.0×10^{-2}	59.1
25	4.6×10^{-6} - 1.0×10^{-2}	58.3
30	8.5×10^{-5} - 1.0×10^{-2}	57.2

Table 4. The effect of plasticizers on the performance of sensor no. 4

Plasticizer *(%)	Working rage (M)	Detection limit (M) ± **S.D	Slope(mV/decade of activity) ± **S.D	Response time (s)
DOP (40%)	$6.5 \times 10^{-5} - 1.0 \times 10^{-2}$	$5.5 \times 10^{-5} \pm 0.07$	53.6 ± 0.05	18
DOP (45%)	$4.7 \times 10^{-5} - 1.0 \times 10^{-2}$	$4.3 \times 10^{-5} \pm 0.05$	54.4 ± 0.06	17
DOP (49%)	$3.3 \times 10^{-5} - 1.0 \times 10^{-2}$	$2.8 \times 10^{-5} \pm 0.06$	55.6 ± 0.04	17
DOP (50%)	$4.2 \times 10^{-5} - 1.0 \times 10^{-2}$	$3.5 \times 10^{-5} \pm 0.05$	54.6 ± 0.04	19
CN (40%)	$3.5 \times 10^{-5} - 1.0 \times 10^{-2}$	$2.8 \times 10^{-5} \pm 0.06$	55.5 ± 0.05	15
CN (45%)	$2.5 \times 10^{-5} - 1.0 \times 10^{-2}$	$1.9 \times 10^{-5} \pm 0.05$	56.4 ± 0.06	14
CN (49%)	$1.3 \times 10^{-5} - 1.0 \times 10^{-2}$	$1.0 \times 10^{-5} \pm 0.05$	57.5 ± 0.06	14
CN (50%)	$1.8 \times 10^{-5} - 1.0 \times 10^{-2}$	$1.4 \times 10^{-5} \pm 0.05$	56.3 ± 0.04	15
DBBP (40%)	$7.4 \times 10^{-5} - 1.0 \times 10^{-2}$	$6.9 \times 10^{-5} \pm 0.07$	52.4 ± 0.05	19
DBBP (45%)	$6.8 \times 10^{-5} - 1.0 \times 10^{-2}$	$6.4 \times 10^{-5} \pm 0.06$	53.8 ± 0.04	18
DBBP (49%)	$6.3 \times 10^{-5} - 1.0 \times 10^{-2}$	$5.9 \times 10^{-5} \pm 0.06$	54.4 ± 0.03	18
DBBP (50%)	$6.6 \times 10^{-5} - 1.0 \times 10^{-2}$	$6.1 \times 10^{-5} \pm 0.04$	53.9 ± 0.05	21
o-NPOE (40%)	$2.6 \times 10^{-6} - 1.0 \times 10^{-2}$	$1.9 \times 10^{-6} \pm 0.03$	58.2 ± 0.07	13
o-NPOE (45%)	$2.4 \times 10^{-6} - 1.0 \times 10^{-2}$	$1.4 \times 10^{-6} \pm 0.03$	58.9 ± 0.06	12
o-NPOE (49%)	$2.2 \times 10^{-6} - 1.0 \times 10^{-2}$	$1.2 \times 10^{-6} \pm 0.03$	59.2 ± 0.06	12
o-NPOE (50%)	$2.5 \times 10^{-6} - 1.0 \times 10^{-2}$	$1.5 \times 10^{-6} \pm 0.03$	58.6 ± 0.05	14

*The % (w/w) of total composition of each plasticizer in sensor no. 4

**SD, Standard deviation for three consecutive measurements.

Table 5. Determination of desipramine hydrochloride using sensor No. 4 in pharmaceutical preparation.

Drug		μg/50 mL^{-1} (Aqueous)	
	Taken	% Found ± SD*	RSD (%)
Desipramine hydrochloride (50 mg)	10	9.92 ± 0.06	0.60
	20	19.91 ± 0.04	0.45
	30	29.89 ± 0.06	0.23
	40	39.86 ± 0.05	0.25

*Average of five measurements

Table 6. The comparative evaluation of results with LC.

Serial No.	Time (h)*	Proposed Sensor No. 4 (gm/mL)		LC** (gm/mL)	
		Blood plasma ± SD	***Urine ± SD	Blood plasma ± SD	***Urine ± SD
1	6	$5.4 \times 10^{-6} \pm 0.43$	$5.2 \times 10^{-6} \pm 0.44$	$4.7 \times 10^{-6} \pm 0.63$	$3.8 \times 10^{-6} \pm 0.52$
2	8	$4.4 \times 10^{-6} \pm 0.62$	$4.1 \times 10^{-6} \pm 0.53$	$4.4 \times 10^{-6} \pm 0.54$	$3.3 \times 10^{-6} \pm 0.54$
3	10	$3.8 \times 10^{-6} \pm 0.33$	$3.4 \times 10^{-6} \pm 0.46$	$4.0 \times 10^{-6} \pm 0.48$	$3.0 \times 10^{-6} \pm 0.62$
4	12	$3.1 \times 10^{-6} \pm 0.42$	$2.8 \times 10^{-6} \pm 0.51$	$3.8 \times 10^{-6} \pm 0.45$	$2.6 \times 10^{-7} \pm 0.44$
5	14	$2.7 \times 10^{-6} \pm 0.52$	$2.4 \times 10^{-6} \pm 0.46$	$3.4 \times 10^{-6} \pm 0.62$	$2.3 \times 10^{-6} \pm 0.62$
6	16	$2.3 \times 10^{-6} \pm 0.47$	$1.8 \times 10^{-6} \pm 0.52$	$3.0 \times 10^{-6} \pm 0.52$	$2.0 \times 10^{-6} \pm 0.67$
7	18	$1.6 \times 10^{-6} \pm 0.51$	$1.2 \times 10^{-6} \pm 0.42$	$2.4 \times 10^{-6} \pm 0.44$	$1.4 \times 10^{-6} \pm 0.41$

*Subjects had a mean ± SD age of 31.5±10.8 years

**YMC Pack Pro C_{18} column (150 × 4.6 mm and 3.0 µm particle size)

***The urine sample has a lower availability of desipramine hydrochloride, as it is maximally metabolized.

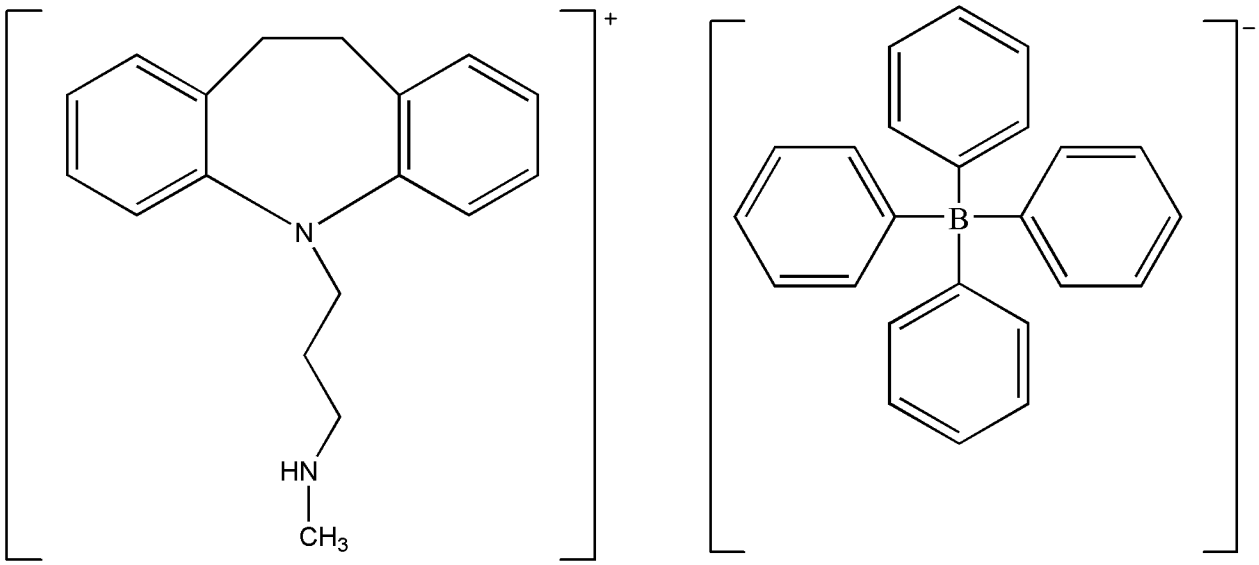

Figure 1.

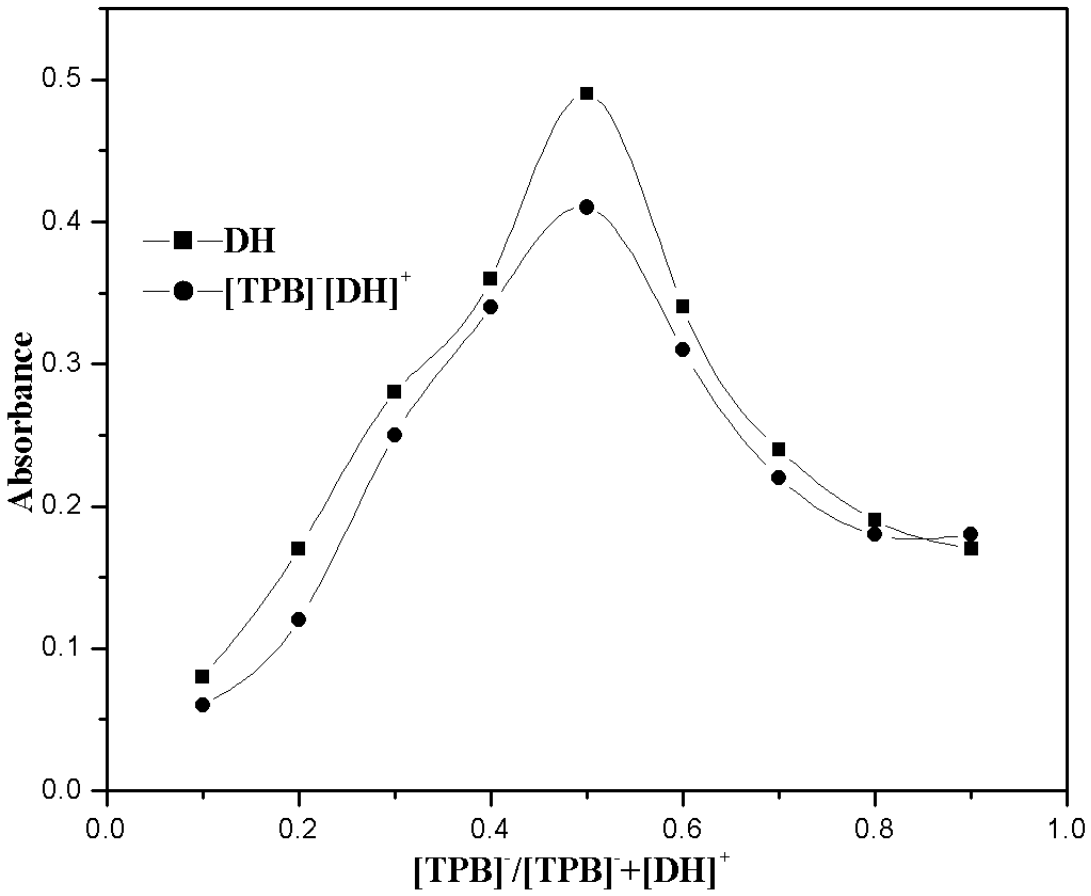

Figure 2.

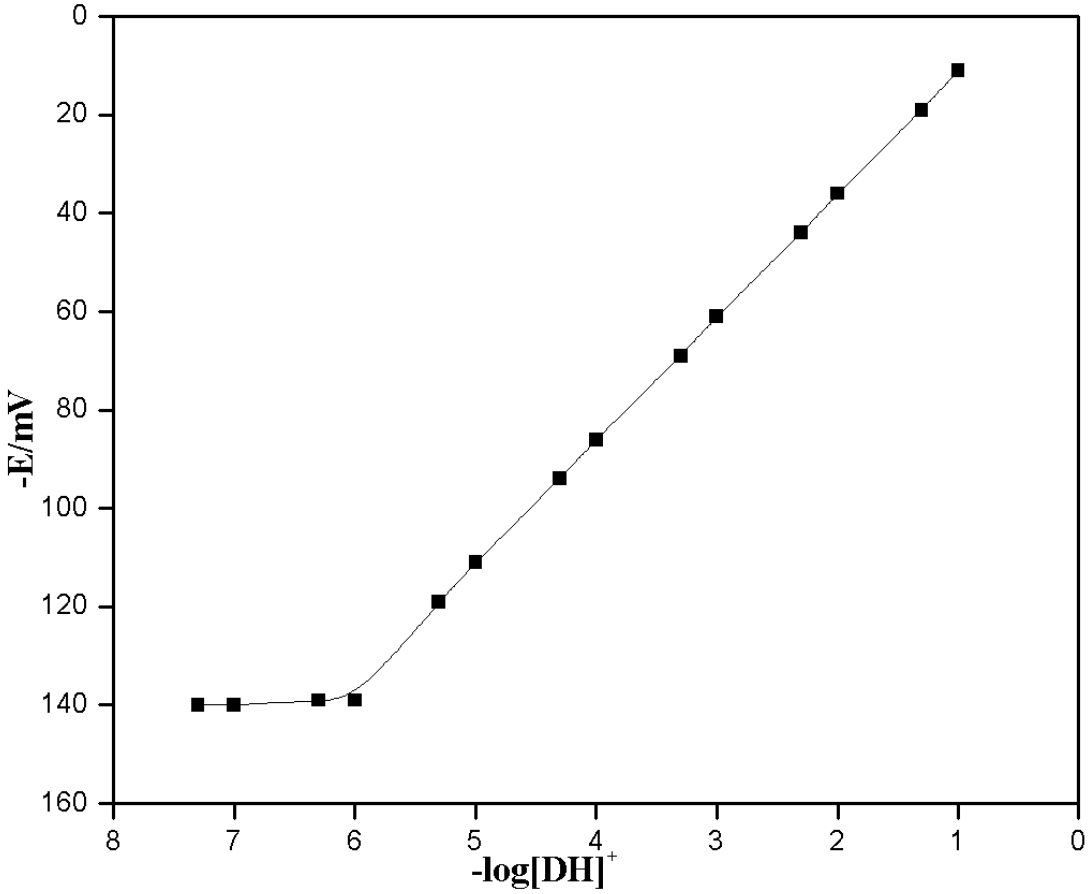

Figure 3.

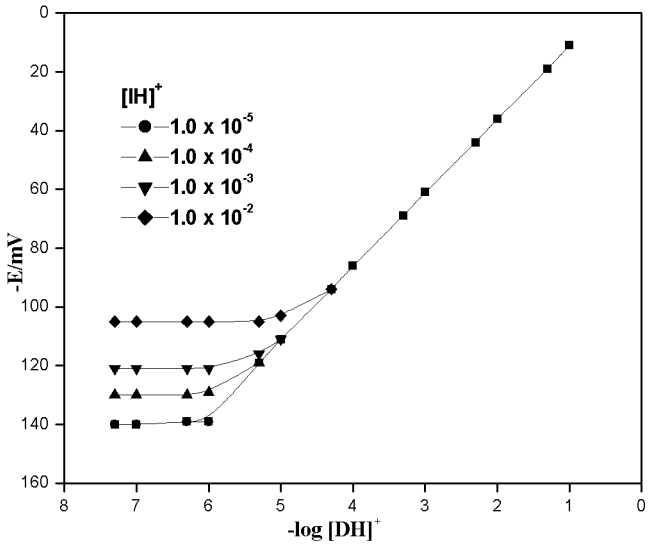

Figure 4.

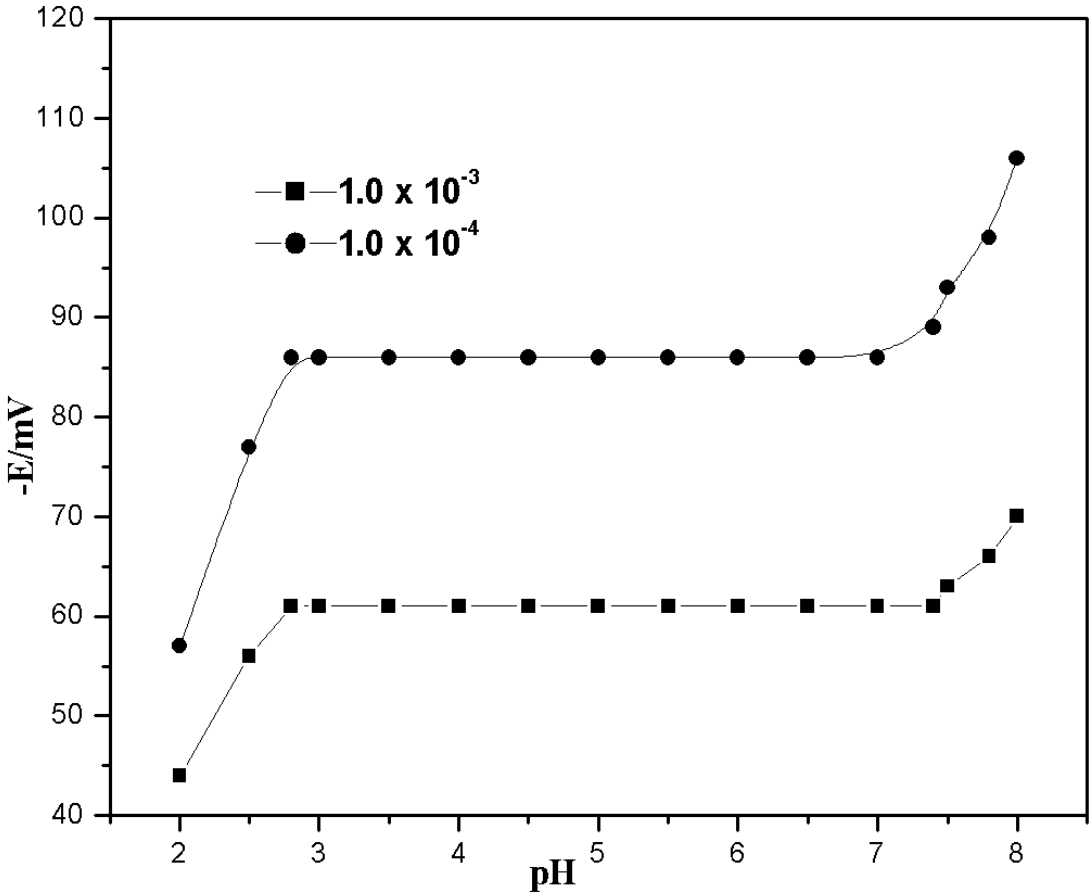

Figure 5.