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Effect of Plasticizers and Ion-Exchangers on the Detection Limit of Tramadol-PVC Membrane Electrodes

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Abstract

The detection limit of tramadol hydrochloride was effectively improved by proper selection of a plasticizer and ion exchanger. Two plastic membrane electrodes for the determination of tramadol hydrochloride (TDCl) were fabricated and fully characterized in terms of composition, life span, usable pH range and working concentration range. The membranes of these electrodes consist of an ion-exchanger such as tramadolium-silicotungstate (TD-ST), silicomolybdate (TD-SM), dispersed in PVC matrix with different plasticizers, namely 2-nitrophenyl octyl ether (2-NPOE), dioctyl phthalate (DOP), dibutyl phthalate (DBP), tris(2-ethylhexyl) phosphate (TEPh), dioctyl sebacate (DOS), tributyl phosphate (TBPh) and dibutyl butyl phosphonate (DBBPh). Experiments showed that DBP was the best plasticizer and TD-PT The best ion pair for their combination produced the lowest detection limit. The present electrodes show clear discrimination of tramadol hydrochloride from several inorganic, organic ions, sugars and some common drug excipients. The sensors were applied for determination of tramadol hydrochloride in urine, milk and pharmaceutical preparations using potentiometric determination, standard addition and the calibration curve methods. The results obtained were satisfactory with excellent percentage recovery comparable and sometimes better than those obtained by other routine methods for the assay.

Keywords:

Ion-selective electrode; ion-association; PVC membrane electrode; tramadol hydrochloride; biological fluids

1. Introduction

Tramadol hydrochloride ((±) *trans*-2-[(dimethylamino) methyl]-1-(3- methoxyphenyl)-cyclohexanol) is a synthetic, centrally acting, analgesic agent (Fig. 1), used for relief of moderate to chronic pain and has no clinically relevant cardiovascular or respiratory depressant activity. Furthermore, it does not have a prostaglandin inhibitory effect. The dosage of tramadol should be adjusted to the intensity of pain and to the response of an individual patient (Its therapeutic plasma concentration is in the range of 100–300 ng L^{-1}). Tramadol is rapidly and almost completely absorbed after oral administration but its absolute bioavailability is only 65–70% due to first-pass metabolism. Approximately 10–30% of the parent drug is excreted unmetabolized in the urine [1].

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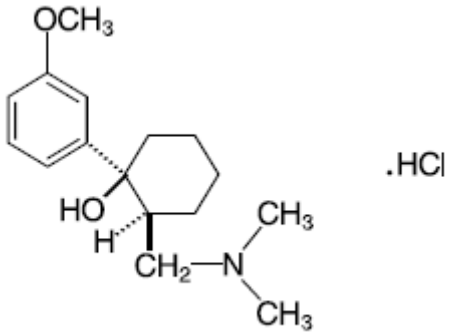


Fig. 1. The chemical structure of tramadol hydrochloride

The development and application of ion-selective electrodes for pharmaceutical analysis continue to be of interest because these sensors offer the advantage of simple design and operation, reasonable selectivity, fast response, applicability to colored and turbid solutions and possible interface with automated and computerized systems [2,3]. For these advantages, ISEs found various applications: in clinical chemistry, environmental protection, water, soil, etc… and analytical chemistry in general [4].

Several analytical methods were described for the determination of tramadol in biological and pharmaceutical samples involve a number of high-performance liquid chromatographic (HPLC) [5], electrochemical [6], spectrophotometric [7] and potentiometric methods [8-11].

Many of these methods involve several time-consuming manipulations, extraction steps, derivatization reactions that are liable to various interferences as well as being not applicable to colored and turbid solutions. There are a few reports of potentiometric ISEs based on PVC membrane [8-11]. However, these electrodes are not very fruitful as they have either one, two, or in some cases, all of the following problems: 1) a high detection limit ≈ 1.0 x 10^{-5} mol L^{-1}) a narrow working concentration range 3) a long response time 4) serious interferences from various cations.

Cleary, there is an important need for a recipe that makes the fabrication and optimization of potentiometric sensors with low detection limit, high stability and reproducibility a straightforward process.

In recent decades, many intensive studies on the design and synthesis of sensitive ion-carriers as sensory molecules for ion-selective electrodes have been reported. Heteropoly acids (silicotungstic, silicomolybdic acids) are promising candidates for the formation of highly insoluble ion-associates with many organic cations [12, 13].

In this work we present the results of an attempt to lower the detection limit of tramadol sensors by using various ion-pairs and various plasticizers with miscellaneous properties and different concentration of internal solution. Each of these sensors incorporates an ion-association of the tramadol cation (TD^+) with silicotungstate, silicomolybdate anion as electroactive material in PVC matrix membrane plasticized with dibutyl phthalate (DPB). It is clear that using these plasticizers and ion-pairs made it possible to attain results that are much better than those reported. Namely, the detection limit was lowered to 2.1 x 10^{-6} and 5.3 x 10^{-6} mol L^{-1} and the concentration range was 5.5 x 10^{-6}-1.0 x 10^{-1} and 7.3 x 10^{-6}-1.0 x 10^{-1} mol L^{-1}

2. Experimental

2.1. Reagents

Tramadol hydrochloride TDCl was obtained from Pharmacare LTD company (Ramallah- Palestine). The pharmaceutical preparations containing TDCl (Tramal, tablets, capsules, drops and ampoules) were obtained from local drug stores. Graphite powder, 2-nitrophenyl octyl ether (2-NPOE), dioctyl phthalate (DOP), dibutyl phthalate (DBP), tris(2-ethylhexyl) phosphate (TEPh), dioctyl sebacate (DOS), tributyl phosphate (TBPh) and dibutyl butyl phosphonate (DBBPh) as well as metal salts were purchased from Aldrich and used as received. Silicotungstic acid (STA) and silicomolybdic acid (SMA) were obtained from Sigma.

2.2. Apparatus

Potentiometric measurements were carried out with a digital millivoltmeter (SR-MUL-3800). pH measurements were made with a digital pH meter (Wissenschaftlich-Technische Werkstatten GmbH (WTW)- Germany) under stirring conditions at room temperature (25.0±1.0 $^{\circ}$C)

The performance of the electrodes was investigated by measuring the emfs of TD solutions with a concentration range of 10^{-7}– 10^{-1} mol L^{-1} by serial dilution. Each solution was stirred and the potential reading was recorded when it became stable, and plotted as a logarithmic function of TD cation activities which are calculated according to the Debye-Hückel equation

$$\log \gamma = - 0.511\ Z^2\ [\mu^{1/2}(1+1.5\ \mu^{1/2}) - 0.2\ \mu]$$

which is applicable to any ion, where μ is the ionic strength and Z the charge [14].

2.3. Preparation of ion-pair

An ion-pair was made from tramadol hydrochloride (TD) and one of the following substances silicotungstic acid (STA) and silicomolybdic acid (SMA) according to a previously reported method [15]. These ion-pairs were used as the active substances for preparing the PVC membrane electrodes of tramadol hydrochloride.

2.4. Preparation of the electrodes

The membranes were prepared as previously described by Thomas and co-workers [16]. The resulting membranes were then sectioned with a cork borer and mounted across the opening of the PVC tube of about 7 mm i.d. and 1.5 cm length using a glue of PVC in THF. The electrodes TD-ST and TD-SM were filled with a solution that is 10^{-1} mol L^{-1} NaCl and 10^{-3} mol L^{-1} TDCl and preconditioned by soaking in 10^{-3} mol L^{-1} TDCl for 15 minutes.

2.5. Selectivity coefficient determination

The separate solution method (SSM) and the Matched Potential Method (MPM) [17] are employed to determine the selectivity coefficients $log\, K^{pot}_{Drug,J^{Z+}}$ of the potentiometric sensors towards different species.

In the SSM, the potential of a cell comprising a working electrode and a reference electrode is measured in two separate solutions, one containing the drug ions, E_1, and the other containing the interferent ions (J), E_2, and S is the slope of the calibration graph.

These values were used to calculate the selectivity coefficient, $log\, K^{pot}_{Drug,J^{Z+}}$ from the following equation:

$$log\, K^{pot}_{Drug,J^{Z+}} = \frac{E_2 - E_1}{S} + log[\,Drug\,] - log[\,J^{Z+}\,]^{1/Z}$$

In MPM, specified amounts of TDCl in the range of $2x10^{-4}$ to $2x10^{-5}$ mol L^{-1} were added to a reference solution of TDCl, and the corresponding potential change (ΔE) was measured. In a separate experiment, the interfering ion (J) (in the range of 1.0 x 10^{-1} – 1.0 x 10^{-2} mol L^{-1}) was successively added to an identical reference solution until the change in potential matched the ΔE value. The values of $log\, K^{pot}_{Drug,J^{Z+}}$ were then calculated using the following equation:

$$log\, K^{pot}_{Drug,J^{Z+}} = \frac{a_{Drug}}{a_J}$$

Where the a_J is the activity of the added interferent.

2.6. Sample preparation

Tramadol hydrochloride was determined in different formulations (100 mg TDCl/capsule, 100 mg TDCl/tablet, 100 mg TDCl/2 mL-ampoule and 100 mg TDCl/1 mL Tramal drops).

Samples of tramadol hydrochloride (ampoules, drops, capsules and tablets) ranging from 4.0 x 10^{-6} to 1.0 x 10^{-3} mol L^{-1} TDCl were determined by the standard addition, potentiometric titration, and the calibration curve methods respectively. 1.5 mL-ampoule or 5 mL of drops solution were transferred to a 50 mL volumetric flask and diluted to the mark with distilled water. 3 tablets or capsules were powdered and homogenized as described previously [28, 29]. A portion of the powdered mass equivalent to about 150.0 mg of TDCl was accurately weighed and dissolved in 50 mL of distilled water. This procedure produced 0.01 M solutions of tramadol in these preparations (ampoules, drops and tablets or capsules). Different volumes of these solutions (0.02–8.0 mL equivalent to 4.0 x 10^{-6} to 1.4 x 10^{-3} mol L^{-1}) were taken and analysed by the above methods using the present electrodes. Each analysis was repeated 5 times.

2.7. Sample analysis

The standard addition method in which small increments (10-100 μL) of (0.1 mol L^{-1}) TDCl solution were added to 50.0 mL aliquot-samples of various concentrations (4.0 x 10^{-6} to 1.0 x 10^{-3} mol L^{-1}) TDCl was applied. The change in potential at (25 ± 0.1°C) was recorded after each increment and these data were used to calculate the concentration of TDCl in the drug samples using the following equation.

$$C_X = \frac{C_S x V_S}{(V_X + V_S)10^{\Delta E/S} - V_X}$$

where C_x is tramadol concentration in the testing sample, C_s is the concentration of the standard, V_x and V_s are the corresponding volumes, S is the slope of the electrode response, and ΔE is the change in potential [18].

The potentiometric titration of different volumes of 1.0 x 10^{-3} M and 1.0 x 10^{-2} mol L^{-1} TDCl solution: 3-10 mL equivalent to 0.9 -30 mg, were transferred to a 25 mL beaker, and titrated with a standard solution of Na-TPB using the prepared TD-electrodes as indicator electrodes. The end points were determined from the S-shaped curve.

In the calibration graph method, different amounts of TDCl were added to 50 mL of water comprising a concentration range from 1.0 x 10^{-7} to 1.0 x 10^{-1} mol L^{-1} and the measured potential was recorded using the present electrode. Data were plotted as potential versus logarithm of the TD^{+} activity and the resulting graph was used for subsequent determination of unknown drug concentration [19].

2.8. Analysis of spiked urine and milk samples

The samples (5 mL of urine and 10 mL of humanized cow milk) were spiked with tramadol hydrochloride and left stirred for 5 min, transferred to a 25-mL volumetric flask and completed to the mark with distilled water to give 4.0×10^{-6} to 1.0×10^{-3} mol L^{-1} TDCl. These solutions were subjected to the standard additions method or the calibration graph method for TDCl determination [20].

3. Results and Discussion

The sensitivity and selectivity of any membrane electrode is significantly related to the composition of the ion-selective membrane, the nature and amount of the solvent mediators used. Therefore it is necessary to study their effect on the behavior of the proposed electrode. The effect of the nature and the amount of the plasticizers and the ion associates on the potential response of the proposed tramadol selective electrodes were investigated. The results are provided in Table 1.

3.1. Ion-pair selection

Ion-exchangers used in ion-selective membrane sensors should have rapid exchange kinetics and adequate stability. In addition, they should have appreciable solubility in the membrane matrix and have sufficient lipophilicity to prevent leaching from the membrane into the sample solution [21]. The ion-exchanger incorporated in each electrode was an ion-association of the drug cation with a heteropoly anion: silicotungstate $H_4(SiW_{12}O_{40})$ or silicomolybdate $H_4(SiMo_{12}O_{40})$. These species with high molecular weights anions, 1823 and 2880 respectively, have different lipophilicities and stabilities. They were used as electroactive materials in plasticized poly(vinyl chloride) matrix membranes, and were candidates for the formation of highly lipophilic ion associates with many organic cations as well as active the recognition elements in the proposed electrodes [22]. A few membranes with miscellaneous compositions were made and tested. The membrane containing 0.5% of the ion-pair produced the best response as shown in table 1. Higher ratios (> 0.5%) were insoluble in THF. Membranes with no ion-exchanger have lower sensitivity and selectivity with poor repeatability.

The potentiometric response of the electrodes prepared with the optimum amounts of TD_4ST are TD_4SM was examined in the concentration range 1.0×10^{-7} to 1.0×10^{-1} mol L^{-1} TDCl solutions. The calibration plots for the electrodes are represented in Fig. 2, which show linearity over the concentration range of 5.5×10^{-6} to 1.0×10^{-1} M and 7.3×10^{-6} to 1.0×10^{-1} mol L^{-1} and limits of detection were between 2.1 $\times10^{-6}$ and 5.3 $\times10^{-6}$ mol L^{-1} for sensors TD-ST and TD-SM, respectively. The response characteristics of the electrodes were systematically evaluated according to the IUPAC recommendations [18] and summarized in Table 2.

Table 1. Composition and slope of calibration curves for TDCl membrane electrodes at 25.0±0.1 °C.

Composition (%)			S	C.R.	LOD	R.S.D	$R_{(s)}$
I.P	PVC	Plasticizer					
TD-ST							
--	47.5	52.5(DBP)	53	$3.1x10^{-5}$-$2.0x10^{-2}$	$1.2x10^{-5}$	2.32	18
0.1	46.9	53.0(DBP)	57	$8.8x10^{-6}$-$1.0x10^{-2}$	$5.8x10^{-6}$	1.21	10
0.3	46.5	53.2(DBP)	60	$8.6x10^{-6}$-$1.0x10^{-2}$	$5.1x10^{-6}$	1.02	10
*0.5	46.5	53.0(DBP)	61	$5.5x10^{-6}$-$1.0x10^{-1}$	$2.1x10^{-6}$	0.88	5
0.5	46.5	53.0(2-NPOE)	56	$7.8x10^{-6}$-$1.0x10^{-1}$	$4.5x10^{-6}$	1.10	13
0.5	46.5	53.0(DOP)	57	$6.6x10^{-6}$-$1.0x10^{-1}$	$3.8x10^{-6}$	0.87	7
0.5	46.5	53.0(DOS)	55	$7.7x10^{-6}$-$1.0x10^{-1}$	$5.2\ x10^{-6}$	0.43	12
0.5	46.5	53.0(TEPh)	57	$9.2x10^{-6}$-$1.0x10^{-1}$	$6.3x10^{-6}$	0.79	10
0.5	46.5	53.0(DBBPh)	41	$7.5x10^{-5}$-$1.0x10^{-1}$	$5.5x10^{-5}$	1.51	23
TD-SM							
--	47.5	52.5(DBP)	51	$5.1x10^{-5}$-$2.0x10^{-2}$	$3.2x10^{-5}$	2.85	23
0.1	46.9	53.0(DBP)	57	$1.8x10^{-5}$-$1.0x10^{-2}$	$6.8x10^{-6}$	1.55	10
0.3	46.5	53.2(DBP)	59	$8.8x10^{-6}$-$3.0x10^{-2}$	$5.9x10^{-6}$	1.01	12
*0.5	46.5	53.0(DBP)	60	$7.3x10^{-6}$-$1.0x10^{-1}$	$5.3x10^{-6}$	0.55	5
0.5	46.5	53.0(2-NPOE)	55	$8.5x10^{-6}$-$1.0x10^{-1}$	$6.9x10^{-6}$	1.10	12
0.5	46.5	53.0(DOP)	56	$9.8x10^{-6}$-$1.0x10^{-1}$	$7.5x10^{-6}$	1.87	15
0.5	46.5	53.0(DOS)	52	$1.6x10^{-5}$-$1.0x10^{-1}$	$8.3x10^{-6}$	1.02	20
0.5	46.5	53.0(TEPh)	56	$1.5x10^{-5}$-$1.0x10^{-2}$	$8.7x10^{-6}$	1.88	12
0.5	46.5	53.0(DBBPh)	44	$9.6x10^{-5}$-$1.0x10^{-2}$	$5.9\ x10^{-5}$	2.19	25

I.P: Ion-pair, S: slope (mV/decade), C.R.: concentration range (M), LOD: limit of detection, $R_{(s)}$: response time(s)

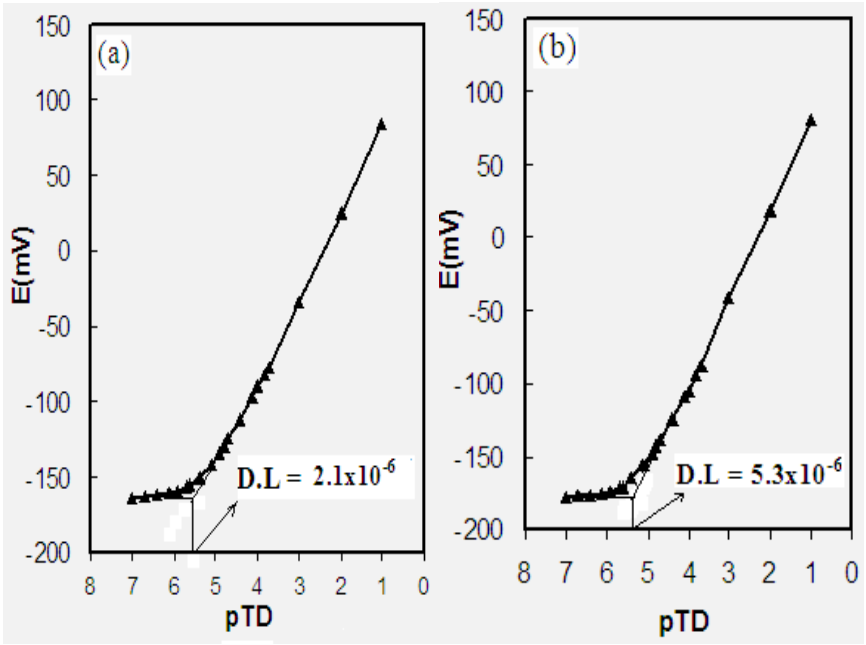


Fig. 2. The calibration curve and the detection limit value for electrods a) TD-ST b) TD-SM

Table 2. Response characteristics of TD-ST and TD-SM electrodes

Parameter	TD.ST electrode	TD.SM electrode
Composition (%) (ion-associate-PVC-DBP)	0.5-46.5-53.0	0.5-46.5-53.0
Slope, (mV/decade)	61.0±0.5	60.0±0.5
Linearity range (mol L^{-1})	5.5 x 10^{-6} - 1.0 x 10^{-1}	7.3 x 10^{-6} - 1.0 x 10^{-1}
Limit of detection (mol L^{-1})	2.1 x 10^{-6}	5.3 x 10^{-6}
Working pH range	1.5-6.5	1.5-6.3
Response time (s)	≤ 5-8	≤ 7-10
Life span (days)	33	23

3.2. Plasticizer selection

Plasticizers must fulfill the four principal criteria mentioned earlier: high lipophilicity, solubility in the polymeric membrane (no crystallization) as well as no exudation (one phase system) and good selectivity behaviour of the resulting membrane [23].With these characteristics, the addition of plasticizer improves the sensitivity and stability of the sensor for it has a dual function: it acts both as a liquefying agent, enabling the homogenous solubilization of the membrane ionophore, and modifying the distribution constant of the ionophore used. The proportion of the plasticizer used must be optimized in order to minimize the electrical asymmetry of the membrane, to keep the sensor as clean as possible, and to stop leaching to the aqueous phase [24]. Furthermore, the nature of the plasticizer affects both the dielectric constant of the membrane and the mobility of the ionophore and its complex [25]. Therefore, the influence of the polarity of the plasticizer on the cation selectivity of the membrane was investigated. The plasticizers viz. (2-NPOE), (DOP), (DBP), (TEPh), (DOS), (TBPh) and (DBBPh) with different properties were employed to study the effect on the electrochemical behavior of the electrodes.

Generally, plasticizers improve certain characteristics of the electrodes. However, some plasticizers affect the response characteristics adversely depending on some physical parameters such as the dielectric constant (ε), lipophilicity (Log P_{TLC}), viscosity (η) and molecular weight (M. wt) of the plasticizers used. Still some plasticizers have negligible or even no effect on the response of the electrode. An example of this case is encountered in the present study. Nevertheless, it seems that DBP, with relatively moderate viscosity, lipophilicity, molecular weight and low dielectric constant, produced the best results.

3.3. Effect of Internal Reference Solution

The key to the improvements of detection limit has been the reduction of zero current ion flux effects that enhance the primary ion activities in the sensed sample layer near the membrane surface. This has been achieved by careful adjustment of the composition of the inner solution by reducing ion fluxes in the membrane and by reducing the thickness of the aqueous diffusion layer [26]. Primary ions contaminating the sample in the sensed layer originate either from the membrane or from the internal solution [26]. To investigate the effects of the inner filling solutions on the tramadol ion-selective electrode response, the electrodes prepared were filled with different inner filling solutions (10^{-1} mol L^{-1} NaCl + 10^{-2} mol L^{-1} TDCl, 10^{-1} mol L^{-1} NaCl + 10^{-3} mol L^{-1} TDCl, 10^{-1} mol L^{-1} NaCl + 10^{-4} mol L^{-1} TDCl and 10^{-1} mol L^{-1} NaCl + 10^{-5} mol L^{-1} TDCl) and calibration graphics were plotted for each case. From the viewpoint of linear working range, it was determined that the most

appropriate inner filling solution that could be used to prepare a tramadol- selective electrode was 10^{-1} mol L^{-1} NaCl + 10^{-3} mol L^{-1} TDCl.

3.4. Response time and reversibility and Repeatability of the electrode

The response time [18] of the electrodes was obtained by measuring the time required to achieve a steady state potential (within ±1 mV) after successive immersion of the electrodes in a series of TDCl solutions, each having a 10-fold increase in concentration from 1.0×10^{-5} to 1.0×10^{-1} mol L^{-1}. The electrodes yielded steady potentials within 5–8 s. The potential reading stays constant, to within ±1mV, for at least 5 min. To evaluate the reversibility of the electrode, a similar procedure in the opposite direction was adopted with measurements performed in the sequence of high-to-low sample concentrations. The results, depicted in Fig. 3, clearly indicate that equilibrium is reached in a very short time (~8 s) where measurements were made either from low to high concentrations or vice versa. The response characteristics of the electrodes were systematically evaluated according to the IUPAC recommendations [18] and summarized in Table 2. The repeatability of the potential reading for each electrode was examined by subsequent measurement in 1.0×10^{-3} mol L^{-1} TDCl solution immediately after measuring the first set of solutions at 1.0×10^{-4} mol L^{-1} DcCl. The standard deviation of measuring the electromotive force (emf) for five replicate measurements obtained are 1.44, 1.50 for electrodes A and B respectively in 1.0×10^{-4} mol L^{-1} solution, and 0.45, 0.85 in 1.0×10^{-3} mol L^{-1} solution. This indicates the excellent repeatability of the potential response of the electrodes.

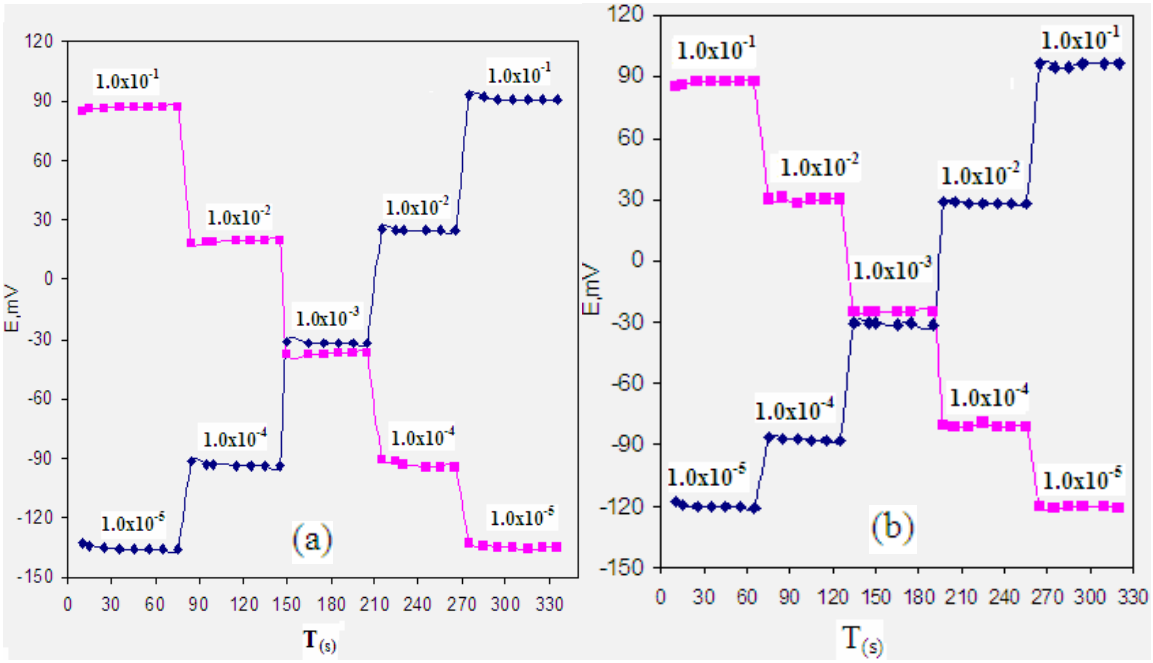
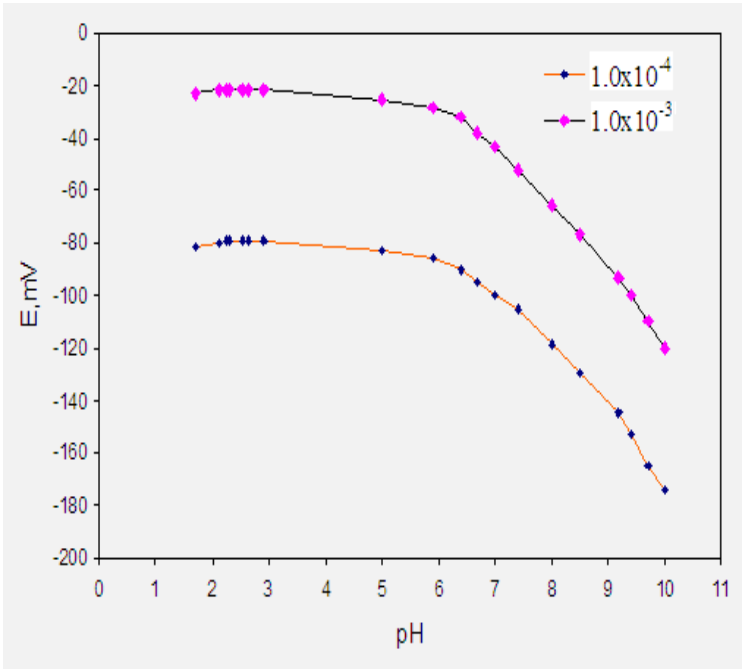


Fig. 3. Dynamic response time of (a) TD.ST electrode and (b) TD-SM electrode for step changes in concentration of TDCl (from low to high and vice versa

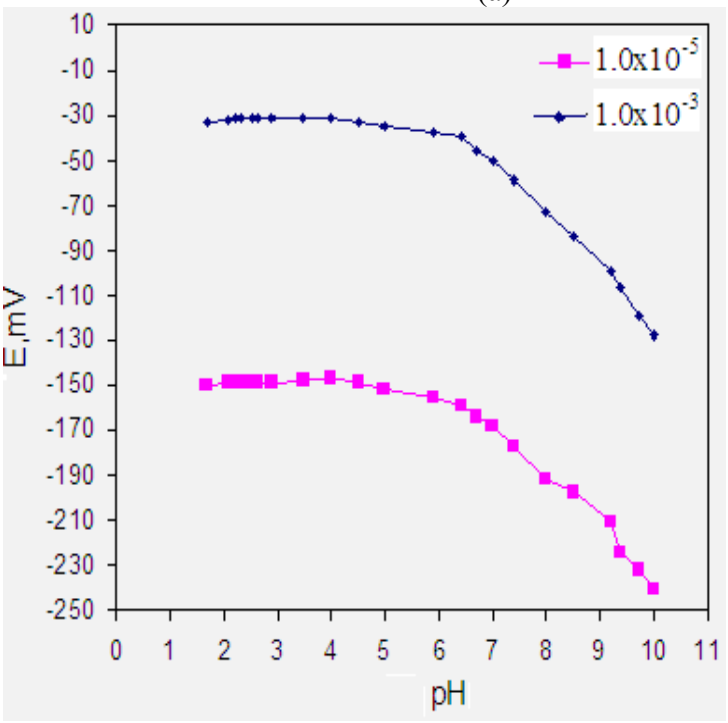
3.5. Effect of pH

The effect of the pH of the test solution on the electrode potentials was studied. The variation in potential with pH changes from 1.0 to 10 was followed by the addition of small volumes of (0.1–1.0 mol L^{-1}) of HCl and NaOH to different concentrations of TDCl solutions. As can be seen from the results shown in Fig. 4, the potential variation due to pH change is

considered acceptable in the pH range 1.5–6.5. Nevertheless, at pH values higher than 6.1, the potential decreases gradually, which can be attributed to the formation of the free tramadol base in the test solution.



(a)



(b)

Fig. 4. Effect of pH of the test solution on the potential response of a) TD-ST b) TD-SM

3.6. Selectivity of the electrodes

The potentiometric selectivity coefficient of an electrode, as one of the most important characteristics, is defined by its relative response for the primary ion over the other ions present in the solution [27]. The separate solution method (SSM) is recommended by IUPAC to determine the selectivity coefficient of the ISE [17]. SSM is based on Nickolsky-Eisenman

equation. However, it has been shown that this method suffers some limitations in terms of the values for ions of unequal charges, a non-Nernstain behavior of interfering ions [28]. Therefore another method named the "matched potential method (MPM)" was recommended especially when the primary ion and/or the interfering ion dissatisfy with the Nernst response or when the involved ions are unequal in charge [29]. The resulting values, presented in Table 4, show that these sensors display significantly high selectivity for tramadol over many common organic and inorganic compounds, drugs, sugars, amino acids as well as some anions.

In pharmaceutical analysis, it is important to test the selectivity toward the excipients and the fillers added to the pharmaceutical preparations. Tramadol pharmaceutical formulations, mainly tablets, contain common excipients such as lactose, glucose, sucrose, starch, stearic acid, magnesium stearate and microcrystalline cellulose. The interference of some of these excipients was explored and measured. It is found that they cause minor effect on the function of the electrode as shown in Table 4. It is worth mentioning that measurements performed on tablets showed accurate results as high as 98% indicating that these excipients made negligible effect on the performance of the electrode.

Comparing the selectivity coefficient values obtained for the investigated electrodes in both SSM and MPM methods collected in Table 3, makes obvious that there is a measurable difference between the values for each interfering ion obtained in both cases. The values of selectivity coefficients obtained using MPM method are more reliable. It is noticed that the results of selectivity tests on interfering monovalent ions are similar to those of tramadol ion. However, the bivalent and trivalent cations produce different results from the two methods. This is reasonable considering that the SSM depends on the charge and gives inaccurate results. However, the MPM gave more accurate ones as it is independent of the charge of the ion.

Table 3. Selectivity coefficient for TD_4ST and TD_3SM

Interfering ions	TD.SM		TD.ST	
	SSM	MPM	SSM	MPM
K^+	6.5×10^{-3}	3.7×10^{-3}	1.2×10^{-3}	2.8×10^{-3}
Na^+	7.7×10^{-3}	2.5×10^{-3}	9.6×10^{-5}	5.5×10^{-3}
Mg^{2+}	2.5×10^{-3}	6.9×10^{-4}	5.4×10^{-4}	8.7×10^{-4}
Ca^{2+}	2.7×10^{-3}	8.5×10^{-4}	9.5×10^{-4}	4.1×10^{-3}
Cu^{2+}	2.2×10^{-3}	3.4×10^{-3}	8.4×10^{-4}	3.5×10^{-3}
Pb^{2+}	7.9×10^{-3}	3.5×10^{-3}	1.9×10^{-3}	1.6×10^{-2}
Ampicilline sodium	2.9×10^{-3}	8.8×10^{-4}	4.5×10^{-4}	3.9×10^{-3}
Diclophinic sodium	$8.1x\ 10^{-3}$	3.6×10^{-3}	2.6×10^{-4}	6.9×10^{-5}
Spiramycine	3.5×10^{-2}	1.9×10^{-2}	1.8×10^{-3}	1.7×10^{-2}
Dioctylsulfosuccinate	9.2×10^{-3}	4.5×10^{-3}	4.5×10^{-4}	9.6×10^{-4}
Spectinomycine Hcl	6.5×10^{-3}	2.7×10^{-3}	8.9×10^{-4}	1.5×10^{-3}
D-Fractose	-	1.5×10^{-4}	-	2.5×10^{-5}
D- Galactose	-	6.0×10^{-5}	-	5.5×10^{-5}
Maltose	-	7.1×10^{-5}	-	6.9×10^{-5}
Glucose	-	1.9×10^{-5}	-	2.8×10^{-5}
Ascorbic acid	1.6×10^{-2}	1.0×10^{-3}	5.4×10^{-4}	4.0×10^{-4}
L-Histidine	6.5×10^{-3}	2.5×10^{-3}	4.5×10^{-4}	3.7×10^{-3}
Glycine	5.3×10^{-3}	8.9×10^{-4}	4.9×10^{-4}	1.9×10^{-4}

3.7. Analytical applications

In order to assess the applicability of the proposed selective electrodes, the methods were applied for determination of tramadol in its pharmaceutical preparations and in different real biological fluids such as urine and milk. The TDCl content in its pharmaceutical preparation (tablets, capsules, drops and ampoules) was determined by the standard additions, the calibration curve and potentiometric titration methods.

It is clear that the amount of TD ion can be a accurately determined with these electrodes as shown in Table 4.

Another analytical application of the proposed sensor is for the determination of tramadol in urine and milk samples. The tramadol content in urine and milk samples was determined with the help of the proposed electrode using the method of standard additions. It is noted that accurate and reproducible results.

The practical utility of the proposed membrane sensors was tested by using as an indicator electrodes for the titration of 5.0 mL of 1.0 x10^{-3} mol L^{-1} TD^{+} ions with a 1.0 x10^{-3} mol L^{-1} of Na-TPB $(C_6H_5)_4BNa$ solution and results are shown in Fig.5. As it is seen, the amount of TD^{+} ions in solution can be accurately determined with the electrode.

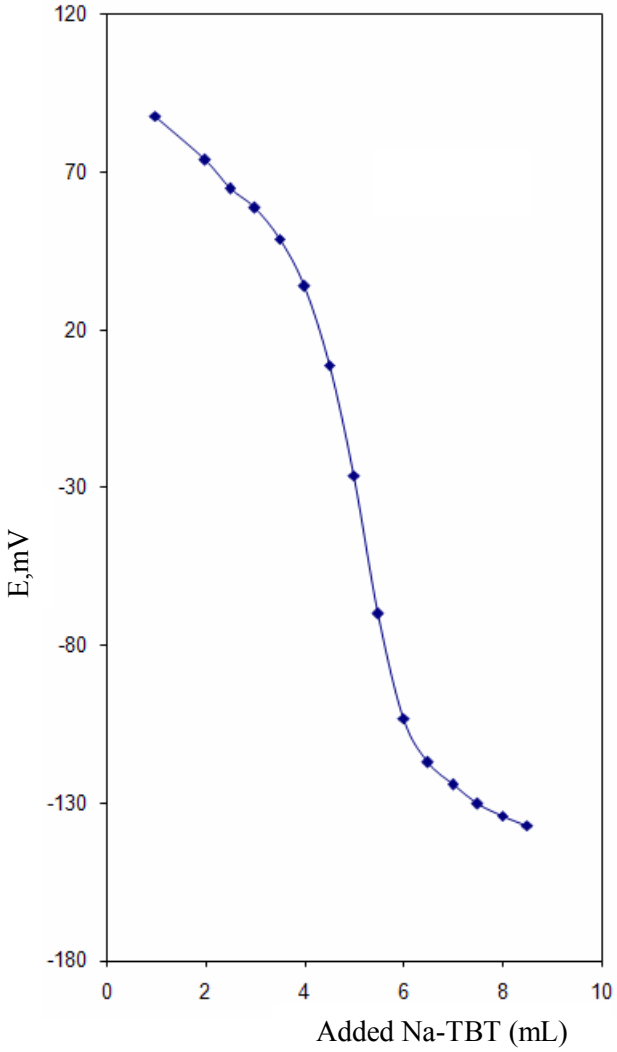


Fig. 5. Potentiometric titration of 5.0 mL of 1.0x10^{-2} mol L^{-1} TDCl with1.0x10^{-2} mol L^{-1} Na-TPB as atitrant using TD.ST electrode

Table 4. Analysis of tramadol in various samples using different methods

Electrode	M		X ± S.E	R.S.D%
	Taken	Found		
TD-ST				
Tablets (Tramal)	P(1.00) x 10^{-3}	(9.85± 0.03) x 10^{-4}	98.5 ± 0.017	0.43
	S(1.00) x 10^{-5}	(9.78± 0.01) x 10^{-6}	97.8± 0.010	0.79
	C(1.00) x 10^{-4}	(1.02 ± 0.03) x 10^{-4}	102.0 ± 0.017	0.43
Capsules (Tramal)	P(1.00) x 10^{-3}	(1.02 ± 0.02) x 10^{-3}	102.0± 0.029	1.32
	S(1.00) x 10^{-5}	(1.01 ± 0.03) x 10^{-5}	101.0± 0.065	1.07
	C(1.00) x 10^{-4}	(9.97 ± 0.02) x 10^{-5}	99.7± 0.009	1.45
Ampoules (Tramal)	P(1.00) x 10^{-3}	(9.91 ± 0.03) x 10^{-4}	99.9 ± 0.017	1.37
	S(1.00) x 10^{-5}	(9.98± 0.01) x 10^{-6}	99.8± 0.010	0.55
	C(1.00) x 10^{-4}	(1.01 ± 0.03) x 10^{-4}	101.0 ± 0.017	0.37
Drops (Tramal)	P(1.00) x 10^{-3}	(1.01± 0.03) x 10^{-3}	101.0± 0.085	1.15
	S(1.00) x 10^{-5}	(9.81 ± 0.02) x 10^{-6}	98.1± 0.025	1.02
	C(1.00) x 10^{-4}	(9.95 ± 0.02) x 10^{-5}	99.5± 0.025	0.66
Urine	S(1.00) x 10^{-5}	(9.91 ± 0.02) x 10^{-6}	99.1± 0.025	1.55
	C(1.00) x 10^{-4}	(1.02 ± 0.02) x 10^{-4}	102.0± 0.025	1.18
Milk	S(1.00) x 10^{-5}	(1.04 ± 0.02) x 10^{-6}	104.0± 0.025	1.38
	C(1.00) x 10^{-4}	(1.03 ± 0.02) x 10^{-4}	103.0± 0.025	1.11
TD-SM				
Tablets (Tramal)	P(1.00) x 10^{-3}	(1.03± 0.03) x 10^{-3}	103.0 ± 0.017	0.69
	S(1.00) x 10^{-5}	(9.78± 0.01) x 10^{-6}	97.8± 0.010	0.88
	C(1.00) x 10^{-4}	(9.82 ± 0.03) x 10^{-5}	98.2 ± 0.017	0.46
Capsules (Tramal)	P(1.00) x 10^{-3}	(1.01 ± 0.02) x 10^{-3}	101.0± 0.039	0.52
	S(1.00) x 10^{-5}	(1.01 ± 0.03) x 10^{-5}	101.0± 0.065	1.44
	C(1.00) x 10^{-4}	(9.97 ± 0.02) x 10^{-5}	99.7± 0.009	1.32
Ampoules (Tramal	P(1.00) x 10^{-3}	(9.87 ± 0.03) x 10^{-4}	98.7 ± 0.057	1.17
	S(1.00) x 10^{-5}	(9.98± 0.01) x 10^{-6}	99.8± 0.010	0.55
	C(1.00) x 10^{-4}	(1.01 ± 0.03) x 10^{-4}	101.0 ± 0.017	0.32
Drops (Tramal)	P(1.00) x 10^{-3}	(1.01± 0.03) x 10^{-3}	101.0± 0.045	1.83
	S(1.00) x 10^{-5}	(9.79 ± 0.02) x 10^{-6}	97.9± 0.025	0.79
	C(1.00) x 10^{-5}	(9.99 ± 0.02) x 10^{-6}	99.9± 0.025	0.35
Urine	S(1.00) x 10^{-5}	(1.04 ± 0.02) x 10^{-5}	104.0± 0.025	1.44
	C(1.00) x 10^{-4}	(1.02 ± 0.02) x 10^{-4}	102.0± 0.025	1.56
Milk	S(1.00) x 10^{-5}	(9.65 ± 0.02) x 10^{-6}	96.5± 0.025	1.72
	C(1.00) x 10^{-4}	(1.03 ± 0.02) x 10^{-4}	103.0± 0.025	1.84

P: potentiometric titration, C: calibration curve, S: standard addition method. The number of replicate measurements = 4.X±S.E.: recovery±standard error. R.S.D, relative standard deviation. The critical value of F = 9.28 and the critical value of t = 3.707.

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