Sensors and Materials, Vol. 28, No. 7 (2016) 797–816
MYU Tokyo

S & M 1239

Carbon Nanotube Electrodes in Comparison to Coated Platinum Wire and Carbon Paste Selective Electrodes for the Determination of Tramadol Hydrochloride in Bulk, Pharmaceutical Formulations, and Spiked Human Plasma and Urine

Nehad A. Abdallah[1,2*]

[1]Pharmacognosy and Pharmaceutical Chemistry Department, Faculty of Pharmacy, Taibah University, Al-Madinah Al-Mounawarah 41477, KSA
[2]Experiments and Advanced Pharmaceutical Research Unit, Faculty of Pharmacy, Ain Shams University, Cairo 1156, Egypt

(Received December 22, 2015; accepted March 28, 2016)

Keywords: tramadol hydrochloride, 18-crown-6-ether, β-cyclodextrin, carbon nanotubes, sensors

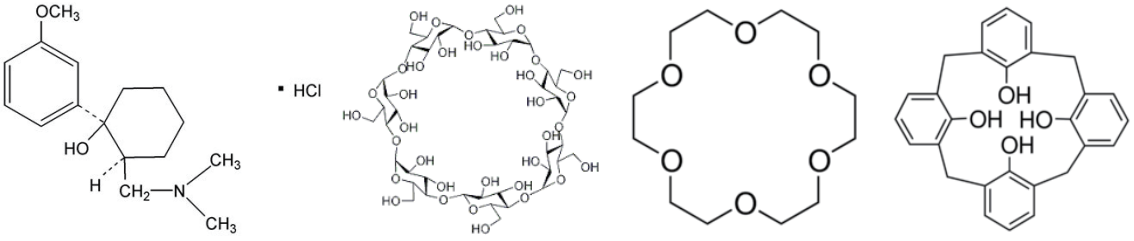
Potentiometric selective electrodes are designed for the electrochemical determination of tramadol hydrochloride (TR.HCl). They are synthesized using coated platinum wire, a carbon paste electrode, and multiwall-carbon-nanotube (MWCNT)-modified carbon paste electrodes. The membranes of the studied electrodes use different types of ionophores as a key element in TR.HCl detection, such as 18-crown-6-ether, β cyclodextrin, and calix[4]arene neutral ionophores. The matrix composition of each electrode is optimized according to ionophore type and concentration, concentration and type of the plasticizer used, and percent of MWCNT added to the carbon paste electrodes. The developed electrodes are completely characterized according to the working concentration range, response time, and suitable pH and temperature range. The developed sensors exhibit excellent stability (more than 8 weeks) with fast dynamic response (<5 s) especially because of the incorporation of MWCNTs which improves the characteristics of the electrodes. The proposed electrodes showed excellent selectivity to TR.HCl in the presence of other related substances. The sensors were applied successfully for the quantitation of TR.HCl in pure form, pharmaceutical dosage form, and in spiked human plasma and urine. They are also applied for quantitation of TR.HCl in dissolution testing of the tablet dosage form.

1. Introduction

Tramadol (1RS,2RS)-2-(dimethylaminomethyl)-1-(3-methoxyphenyl)cyclohexanol is a centrally acting analgesic.(1) The chemical structure of tramadol hydrochloride (TR.HCl) is represented in Fig. 1. It acts on serotonergic and noradrenergic nociception sites. The main tramadol metabolite, O-desmethyl tramadol, acts on the μ-opioid receptor. Its analgesic potency is nearly one tenth that of morphine.(2,3) Tramadol is applied mainly in the treatment of acute (*e.g.*, postoperative, trauma) and chronic (cancer and non-cancer) pain.(4)

*Corresponding author: e-mail: nehad.amin@gmail.com

ISSN 0914-4935



(a) (b) (c) (d)

Fig. 1. Chemical structure of (a) TR.HCl, (b) β-cyclodextrin, (c) 18-crown-6-ether, and (d) calix[4]arene.

Several analytical techniques have been mentioned for the determination of TR.HCl in pharmaceutical dosage forms and in biological fluids, such as spectrophotometry,[5–9] spectrofluorimetery,[10–12] high-performance liquid chromatography,[13–15] thin layer chromatography,[16] and electrochemical sensors.[17–21] The reported TR.HCl potentiometric sensors were based on the formation of an ion association complex between TR.HCl and either tetraphenyl borate, phosphotungestate, or phosphomolybedate for TR.HCl recognition and quantification. They were characterized by their limited linearity range and relatively high detection limit. Researchers have not studied the selectivity of the electrodes in measuring TR.HCl in combined dosage forms with other active pharmaceutical ingredients such as paracetamol.[17–21] None of the reported electrochemical methods mentioned their possible use in measurement of TR.HCl in spiked human plasma samples. Some[17,18,21] studied the determination and measurement of TR.HCl in spiked urine and milk samples.

Carbon nanotubes (CNTs) are electroactive polymers recently used in sensor fabrication owing to their great advantages related to their dimensional and chemical compatibility with different molecules. They possess unique electronic, chemical, and mechanical properties. CNTs possessed sp^2 carbon units several nanometers in diameter and many microns in length. They are characterized by high electronic conductivity for electron transfer reactions and better electrochemical and chemical stabilities in both aqueous and non-aqueous solutions. Additionally, single-wall CNTs and multiwall CNTs (MWCNTs) are also characterized by their high surface area and good electronic properties that make them widely applied in electroanalytical studies.[22]

Ionophores are characterized by their ability to form strong and reversible complexes with a particular ion of interest. The ionophores' chemical structure is characterized by a number of lipophilic groups that minimize the leaching rate from the membrane to the sample phase.[23] This lends more stability and better performance to the membrane relative to the use of ion-association complexes as a key elment for membrane recognition.

Crown ethers (CEs) with their electronegative oxygen atoms (binding site) can form an ion-dipole bond with an ion of interest if the ion size and crown cavity size are matched.[24] Cyclodextrins (CDs) are cyclic oligomers and calixarenes (CXs) are groups of cyclic macromolecules, both of which can form inclusion complexes with organic molecules in their hydrophobic cavities, which are available in different sizes.[25–27] The chemical structures of the ionophores used in this study are shown in Fig. 1.

In the present work, we compared the characteristics of coated platinum wire electrodes (CWEs), carbon paste electrodes (CPEs), and MWCNT-modified carbon-paste electrodes (MWCPEs) using different ionophores for the determination of TR.HCl in pure form, pharmaceutical tablets, and

in spiked human plasma and urine. The proposed sensors are more simple, sensitive, faster, and cheaper than instrumental methods of analysis. Also the sensors provide greater stability and lower detection limits than other reported sensors.

2. Experimental Methods

2.1 Instrumentation

The potentiometric measurements were done using CLEAN PH600 benchtop digital ion analyzer, model 007747 (China) that was attached to an Ag/AgCl double junction reference electrode Z113107-1EA batch 310 (Sigma-Aldrich) filled with 3.0 M KCl saturated with AgCl as the inner filling solution and 10% KNO_3 as the bridge electrolyte. A Heidolph MR Hei-Standard, model 100818877, magnetic stirrer was used during measurements.

2.2 Reagents

The chemicals and reagents used were of analytical grade: tetrahydrofuran (THF) (Fisher Scientific, UK); dioctyl phthalate (DOP), 18-crown-6 (1,4,7,10,13,16-hexaoxyacyclooctadecane) (Acros Organics, USA); propylene glycol, spectroscopic graphite powder (1–2 μm); MWCNT powder (DXL 110–170 nm × 5–9 μm) and calix[4]arene (Aldrich, USA); poly(vinyl chloride) (PVC) carboxylate and β-cyclodextrin (Acros Organics, USA); β-alanine, phosphoric acid, and acetic acid (Fluka Chemie Gmbh, Germany); potassium chloride (Merck, Dermastadt, Germany); sodium chloride, calcium chloride, boric acid, and sodium hydroxide (Prolabo, Pennsylvania, USA); dioctyladipate (DOA) and dibutylphthalate (DBP) (Fluka, USA).

The TR.HCl reference standard was supplied by Mediphar Laboratories, Dbayeh, Lebanon. Its potency is certified to be 99.8%. The paracetamol reference standard was supplied by Mediphar Laboratories, Dbayeh, Lebanon. Its potency is certified to be 99.6%. TR.HCl tablets (Zaldiar®) contained 37.5 mg TR.HCl BN: 00991B and were manufactured by Grunethal GmbH, Aachen, (Germany).

2.3 Procedure

2.3.1 Standard solution preparation

The preparation was conducted at room temperature and was stored at 5 °C during use. A stock solution (1×10^{-1} mol L^{-1}) was prepared by weighing 0.75 g of TR.HCl in a 250 ml volumetric flask, dissolving it, and filling the flask to the mark with deionized water. Working standard solutions (1×10^{-8} to 1×10^{-2} mol L^{-1}) were prepared by suitable dilutions from the stock solution using deionized water.

2.3.2 Fabrication of CWEs

The membranes were prepared by dissolving varying percentages (w/w) of the PVC, ionophores, and DOP in about 10 ml THF. The mixtures were stirred for 15 min untill complete homogeneity was achieved. The petri dishes were covered and left for 1 h to provide slow evaporation of the

solvents, producing a thick homogeneous master PVC membrane coating. An insulated platinum wire (1 mm in diameter and 12 cm in length) was used. The cover at both ends was removed for a length of about 1.5 cm. One end of the wire was immersed in the prepared PVC membrane solution for each membrane separately and allowed to stand for about 15 min to ensure complete air drying and formation of a thin membrane around the wire end. The coated wire membrane sensor was conditioned by soaking in 10^{-2} mol L^{-1} TR.HCl solution for the proper period of time specified for each sensor and stored in the same solution when not in use.

2.3.3 Fabrication of CPEs

The carbon paste electrodes were prepared by proper mixing of the spectroscopic graphite powder (1–2 μm) with the ionophores with DOP as a plasticizer (ratio of graphite powder to DOP was 60:40 w/w for a total weight of components of 0.35 g) in a small mortar until homogenously mixed. The teflon part of the electrode body was filled with the resulting paste. A new surface was obtained by pulling a stainless steel screw forward through the electrode body and polishing the new carbon paste surface with filter paper to obtain a new shiny surface.

2.3.4 Fabrication of MWCPEs

The effect of incorporating MWCNT powder on the behavior of the carbon paste electrode was studied. A previously prepared carbon paste electrode was modified by the addition of variable percentages of carbon nanotubes to the membrane mixture of 0.35 g total weight to the optimum composition obtained for the carbon paste electrode. The mixture was homogenized and the paste was packed into the teflon holder of the electrode body. A fresh surface was obtained by pulling the stainless steel screw forward through the electrode body and polishing the new carbon paste surface with filter paper to obtain a new shiny surface.

2.3.5 Sensors selectivity

The potentiometric selectivity coefficients ($K^{pot}_{A,B}$) of the proposed sensors towards some interfering substances and some co-administered drugs were measured using a separate solution method by applying the following equation[28]

$$\log K^{pot}_{A,B} = [(E_B - E_A)/(2.303RT/Z_AF)] + [1-(Z_A/Z_B)] \log[A],$$

where K^{pot} is the potentiometric selectivity coefficient, E_A is the potential measured for 10^{-3} mol L^{-1} TR.HCl solution, and E_B is the potential measured for 10^{-3} mol L^{-1} interfering solution. The terms Z_A and Z_B are the charges of TR.HCl and the interferent, respectively; $2.303RT/Z_AF$ represents the slope of the calibration plot (mV/concentration decade); and $\log[A]$ is the log of TR.HCl activity.

The electrodes' selectivity coefficients were also measured using the matched potential method.[29,30] This method was based on measurement of potential difference caused by the increase in the TR.HCl activity from 10^{-4} mol L^{-1} to 10^{-3} mol L^{-1}. Then, the ion selective electrodes were placed back into the starting solution, and the interfering ions were added individually until the same potential change was registered. The selectivity coefficient was calculated as the ratio of the respective activity increments resulting in the same potential change.

2.3.6 Potentiometric determination of TR.HCl

The potentiometric determination of TR was carried out using the proposed electrodes by the standard addition method.[31] The change in potential was recorded after the addition of a small addition of standard TR.HCl solution, 1×10^{-2} mol L^{-1}, to 50 ml samples of appropriate concentrations within the linearity range at the appropriate pH value for each electrode. The change in potential reading was recorded for each increment.

2.3.7 Potentiometric determination of TR.HCl in pharmaceutical formulation

Twenty tablets of Zaldiar® were used to determine the TR.HCl concentration in pharmaceutical formulations. Each tablet was accurately weighed, then all tablets were finely powdered together. A portion of the powder tablet equivalent to 0.75 g TR.HCl was weighed and transferred to a 250 ml volumetric flask. Around 100 ml deionized water was added and the flask was sonicated for about 15 min. The solution was filtered and diluted to 250 ml with deionized water to prepare a 1×10^{-1} mol L^{-1} aqueous solution of TR.HCl. Suitable dilutions were prepared to obtain different concentrations from 1×10^{-8} to 1×10^{-2} mol L^{-1} TR.HCl. The potentials of these solutions were measured using the electrodes, and the corresponding concentrations were calculated for each sensor from its specific regression equation.

2.3.8 Potentiometric determination of TR.HCl in spiked human plasma and urine samples

In stoppered tubes, 4.5 ml of human plasma or urine (pH adjusted to 6) was added, then 0.5 ml of 1×10^{-5} to 1×10^{-2} mol L^{-1} of TR.HCl was added, and the tubes were shaken for 1 min. The electrochemical electrodes were immersed in these solutions and then washed with water between measurements. The potential produced for each solution was measured by the proposed sensors, and the concentrations of TR.HCl solutions were determined from the corresponding regression equations.

2.4 Water layer test

The water layer test was performed to show the effect of a water layer between the ion selective membrane and the tranducer.[32] The potential of each of the electrodes was alternately recorded after conditioning in 1×10^{-3} mol L^{-1} TR.HCl solution then 1×10^{-4} mol L^{-1} ephedrine hydrochloride solution and again in 1×10^{-3} mol L^{-1} TR.HCl solution.

3. Results and Discussion

The potentiometric sensors are characterized by their simple design and operation, wide linear dynamic range, relatively fast response, and appropriate selectivity. The ionophores directly affect the response, selectivity, and stability of the electrodes. The ionophores used are characterized by their inner core, which can form inclusion complexes with different molecules with great flexibility.[33]

The incorporation of MWCNT into the membrane composition imparts more advantages to the proposed sensors. They can lower the ohmic resistance and shorten the response time by nearly a

fifth in comparison to the membranes without MWCNT. This is due to the great conductivity of CNTs, which improves the transduction of the signals and enables equilibrium to be attained more rapidly.

It was reported that CNTs possess higher conductivity than graphite, excellent strength, stiffness, and chemical reactivity. This is due to the strength of C–C covalent bond, the surface curvature of the carbon structure, and also from π-orbital misalignment between adjacent pairs of conjugated C atoms. CNTs mediate the electron transfer reaction with the electroactive species in the solution. Their porous structure may also contribute to good wetting properties for the solvents, a better electrode–electrolyte interface, and a large surface area.[34,35]

3.1 Sensor fabrication

Conventional CWEs were prepared using PVC–COOH as a regular support and reproducible trap for ion inclusion complexes. DOP (a non-polar plasticizer) was used to adjust the permittivity and attain the highest possible selectivity and sensitivity of the final membrane. Twelve membranes with different compositions were investigated to determine the optimum percent of each ionophore in addition to the nature and amount of the plasticizer needed to obtain the best performance characteristics. The best composition and performance was found with either 7% β-cyclodextrin with 46.5% DOP (β-CD electrode), 13% 18-crown-6-ether with 43.5% DOP (CE electrode), or 5% calix[4]arene with 47.5% DOP (CX electrode). These membrane compositions yielded the best response with higher slope values of 56.36, 55.32, and 54.33 (mV/concentration decade), respectively, as reported in Tables 1 and 2.

By comparing the performance of CWEs with CPEs and the MWCPEs as reported in Tables 1 and 2, it was very obvious that the performance of CWEs was greatly enhanced by the use of carbon paste membranes especially after the incorporation of MWCNT. As the transduction property of the membrane increased, the response time and the dynamic working range of the membrane improved. The addition of MWCNT greatly enhanced the response time, which reached nearly 3 to 4 s, which in turn increased the sampling rates and detection limit, which can be a great advantage in quality control laboratories. The fast equilibrium attained by the hydrophobic MWCNT hinders the accumulation of water molecules on the electrode surface to facilitate the diffusion of the sensed ion through the electrode surface.[36] It is clear from the results given in Tables 1 and 2 and graphically represented in Figs. 2 and 3 that the incorporation of 5% MWCNT in the membrane matrix decreased the response time, increased the detection limit, improved the slopes that reached 60 mV/concentration decade, and increased the stability of the membranes over 60 d with wider concentration ranges.

In the case of CPEs, it was found that the cyclodextrin electrode (β-CD electrode) with a composition of (7% β-cyclodextrin, 37% DOP, 56% graphite powder), the crown ether electrode (CE electrode) with a composition of (13% 18-crown-6-ether, 35% DOP, 52% graphite powder), and the calixarene electrode (CX electrode) with a composition of (5% calix[4]arene, 38% DOP, 57% graphite powder) exhibited the best performance with slopes of 58.05, 57.66, and 56.08 (mv/concentration decade) with linear concentration ranges of 1.0×10^{-7}–1.0×10^{-2}, 1.0×10^{-6}–1.0×10^{-1}, and 1.0×10^{-8}–1.0×10^{-2} mol L^{-1}, respectively.

In the case of MWCPEs, it was found that the cyclodextrin electrode (β-CD electrode) with a composition of (7% β-cyclodextrin, 37% DOP, 51% graphite powder, 5% MWCNTs), the crown ether electrode (CE electrode), with a composition of (13% 18-crown-6-ether, 35% DOP, 48%

Table 1
Optimizing the composition of CWE, CPE, and MWCPE and their slopes at 25 ± 1°.

Electrode no.	Composition % (w/w)					Slope (mV/decade)	Linearity range (mol L^{-1})	Response time (s)	LOD[d] (mol L^{-1})	RSD%[e]
	PVC–COOH	ionophore	DOP	Graphite powder	MWCNTs					
CWEs										
1	48.5	3% β-CD[a]	48.5	—	—	51.89	1.0×10^{-4}–1.0×10^{-2}	20	4.5×10^{-5}	1.26
2	47.5	5% β-CD	47.5	—	—	55.45	1.0×10^{-4}–1.0×10^{-2}	22	7.0×10^{-5}	1.38
3	**46.5**	**7% β-CD**	**46.5**	—	—	**56.36**	**1.0×10^{-5}–1.0×10^{-2}**	**15**	**3.6×10^{-6}**	**0.98**
4	45	10% β-CD	45	—	—	54.13	1.0×10^{-5}–1.0×10^{-2}	21	1.0×10^{-6}	1.44
5	44	12% β-CD	44	—	—	54.03	1.0×10^{-4}–1.0×10^{-1}	25	6.0×10^{-5}	1.52
6	45	10% CE[b]	45	—	—	50.78	1.0×10^{-4}–1.0×10^{-1}	30	4.0×10^{-5}	1.28
7	**43.5**	**13% CE**	**43.5**	—	—	**55.32**	**1.0×10^{-5}–1.0×10^{-1}**	**17**	**2.0×10^{-7}**	**1.03**
8	42.5	15% CE	42.5	—	—	51.21	1.0×10^{-5}–1.0×10^{-2}	25	1.0×10^{-6}	1.56
9	48.5	3% CX[c]	48.5	—	—	53.11	1.0×10^{-5}–1.0×10^{-2}	30	5.0×10^{-6}	1.42
10	**47.5**	**5% CX**	**47.5**	—	—	**54.33**	**1.0×10^{-6}–1.0×10^{-2}**	**20**	**1.0×10^{-7}**	**0.95**
11	46.5	7% CX	46.5	—	—	50.07	1.0×10^{-5}–1.0×10^{-1}	25	6.8×10^{-6}	1.44
12	45	10% CX	45	—	—	52.39	1.0×10^{-5}–1.0×10^{-2}	25	1.0×10^{-6}	1.39
CPEs										
13	—	5% β-CD	38	57	—	56.31	1.0×10^{-5}–1.0×10^{-2}	16	3.4×10^{-6}	1.53
14	—	6% β-CD	37.5	56.5	—	55.62	1.0×10^{-5}–1.0×10^{-1}	18	2.6×10^{-6}	1.28
15	—	**7% β-CD**	**37**	**56**	—	**58.05**	**1.0×10^{-7}–1.0×10^{-2}**	**9**	**1.0×10^{-8}**	**1.01**
16	—	11% CE	35.5	53.5	—	51.22	1.0×10^{-5}–1.0×10^{-2}	14	8.0×10^{-6}	1.63
17	—	12% CE	35	53	—	53.44	1.0×10^{-6}–1.0×10^{-2}	17	6.7×10^{-7}	1.28
18	—	**13% CE**	**35**	**52**	—	**57.66**	**1.0×10^{-6}–1.0×10^{-1}**	**10**	**1.0×10^{-7}**	**0.89**
19	—	4% CX	38	58	—	54.11	1.0×10^{-6}–1.0×10^{-2}	19	5.8×10^{-7}	1.33
20	—	**5% CX**	**38**	**57**	—	**56.08**	**1.0×10^{-8}–1.0×10^{-2}**	**15**	**4.9×10^{-9}**	**1.04**
21	—	6% CX	37.5	56.5	—	54.29	1.0×10^{-7}–1.0×10^{-2}	22	8.0×10^{-8}	1.28
MWCPEs										
22		7% β-CD	37	53	3	58.77	1.0×10^{-7}–1.0×10^{-1}	7	1.0×10^{-8}	0.98
23		**7% β-CD**	**37**	**51**	**5**	**60.53**	**1.0×10^{-7}–1.0×10^{-1}**	**3**	**1.0×10^{-8}**	**0.88**
24		13% CE	35	50	3	57.47	1.0×10^{-6}–1.0×10^{-2}	8	4.3×10^{-7}	0.84
25		**13% CE**	**35**	**48**	**5**	**59.77**	**1.0×10^{-7}–1.0×10^{-2}**	**3**	**1.0×10^{-8}**	**0.92**
26		5% CX	38	54	3	58.10	1.0×10^{-8}–1.0×10^{-2}	10	5.0×10^{-9}	1.12
27		**5% CX**	**38**	**52**	**5**	**60.6**	**1.0×10^{-8}–1.0×10^{-2}**	**4**	**1.0×10^{-9}**	**0.93**

[a]β-cyclodextrin ionophore.
[b]18-crown-6-ether ionophore.
[c]Calix[4]arene ionophore.
[d]Limit of detection.
[e]Relative standard deviation (calculated using five replicates of each of linear calibration concentrations).

graphite powder, 5% MWCNTs), and the calixarene electrode (CX electrode) with a composition of (5% calix[4]arene, 38% DOP, 52% graphite powder, 5% MWCNTs) exhibited the best performance with slopes of 60.53, 59.77, and 60.60 (mV/concentration decade) with linear concentration ranges of 1.0×10^{-7}–1.0×10^{-1}, 1.0×10^{-7}–1.0×10^{-2}, and 1.0×10^{-8}–1.0×10^{-2} mol L^{-1}, respectively. Figure 4 represents the effect of using different percentages of ionophores on the calibration graphs of the electrodes. The optimum potentiometric calibration profile for the proposed sensors is represented in Fig. 5.

Table 2
Effect of different types of plasticizers on the characteristics of the proposed TR-electrodes.

Electrode type	Plasticizer	Slope (mV/decade)	Linearity range (mol L^{-1})	LOD[d] (mol L^{-1})	RSD%[e]
CWEs					
β-CD[a] electrode	DOA	53.21	1.0×10^{-5}–1.0×10^{-2}	3.6×10^{-6}	1.12
	DOP	56.36	1.0×10^{-5}–1.0×10^{-2}	3.6×10^{-6}	0.98
	DBP	52.42	1.0×10^{-4}–1.0×10^{-2}	4.6×10^{-5}	1.09
CE[b] electrode	DOA	54.12	1.0×10^{-5}–1.0×10^{-1}	3.6×10^{-6}	1.34
	DOP	55.32	1.0×10^{-5}–1.0×10^{-1}	2.0×10^{-7}	1.03
	DBP	51.87	1.0×10^{-5}–1.0×10^{-1}	3.6×10^{-6}	1.52
CX[c] electrode	DOA	52.92	1.0×10^{-5}–1.0×10^{-2}	4.4×10^{-5}	1.22
	DOP	54.33	1.0×10^{-6}–1.0×10^{-2}	1.0×10^{-7}	0.95
	DBP	51.56	1.0×10^{-6}–1.0×10^{-2}	5.6×10^{-6}	1.48
CPEs					
β-CD electrode	DOA	54.06	1.0×10^{-6}–1.0×10^{-2}	4.6×10^{-7}	1.62
	DOP	58.05	1.0×10^{-7}–1.0×10^{-2}	1.0×10^{-8}	1.01
	DBP	56.16	1.0×10^{-5}–1.0×10^{-2}	2.8×10^{-6}	1.41
CE electrode	DOA	55.32	1.0×10^{-6}–1.0×10^{-1}	8.9×10^{-6}	1.80
	DOP	57.66	1.0×10^{-6}–1.0×10^{-1}	2.0×10^{-7}	1.03
	DBP	52.98	1.0×10^{-5}–1.0×10^{-1}	8.3×10^{-6}	1.53
CX electrode	DOA	53.13	1.0×10^{-8}–1.0×10^{-2}	3.6×10^{-8}	1.44
	DOP	56.08	1.0×10^{-8}–1.0×10^{-2}	4.9×10^{-8}	1.04
	DBP	55.08	1.0×10^{-8}–1.0×10^{-2}	3.6×10^{-9}	1.28
MWCPEs					
β-CD electrode	DOA	58.03	1.0×10^{-7}–1.0×10^{-1}	3.6×10^{-8}	1.77
	DOP	60.53	1.0×10^{-7}–1.0×10^{-1}	1.0×10^{-8}	0.88
	DBP	52.67	1.0×10^{-7}–1.0×10^{-1}	5.4×10^{-8}	1.49
CE electrode	DOA	53.45	1.0×10^{-6}–1.0×10^{-2}	5.3×10^{-7}	1.62
	DOP	59.77	1.0×10^{-7}–1.0×10^{-2}	1.0×10^{-8}	0.92
	DBP	57.10	1.0×10^{-7}–1.0×10^{-2}	2.8×10^{-8}	1.73
CX electrode	DOA	55.63	1.0×10^{-8}–1.0×10^{-2}	1.4×10^{-9}	1.95
	DOP	60.6	1.0×10^{-8}–1.0×10^{-2}	1.0×10^{-9}	0.93
	DBP	57.08	1.0×10^{-8}–1.0×10^{-2}	2.7×10^{-9}	1.36

[a]β-cyclodextrin ionophore.
[b]18-crown-6-ether ionophore.
[c]Calix[4]arene ionophore.
[d]Limit of detection.
[e]Relative standard deviation (calculated using five replicates of each of linear calibration concentrations).

3.2 Effect of different plasticizers on electrode performance

The effect of different plasticizers on the performance characteristics of the nine proposed membrane sensors was investigated for three different plasticizers, DOP, DOA, and DBP. The results reported in Table 2 reveal that the use of DOP provides a wide response range, lower detection limits, and stable response with higher slope values in comparison with other plasticizers, perhaps because DOP has a certain lipophilicity and lower polarity that allows better distribution of the ionophores in the membrane matrix. The effect of different plasticizers on the calibration profile of the nine proposed electrodes is graphically represented in Fig. 2.

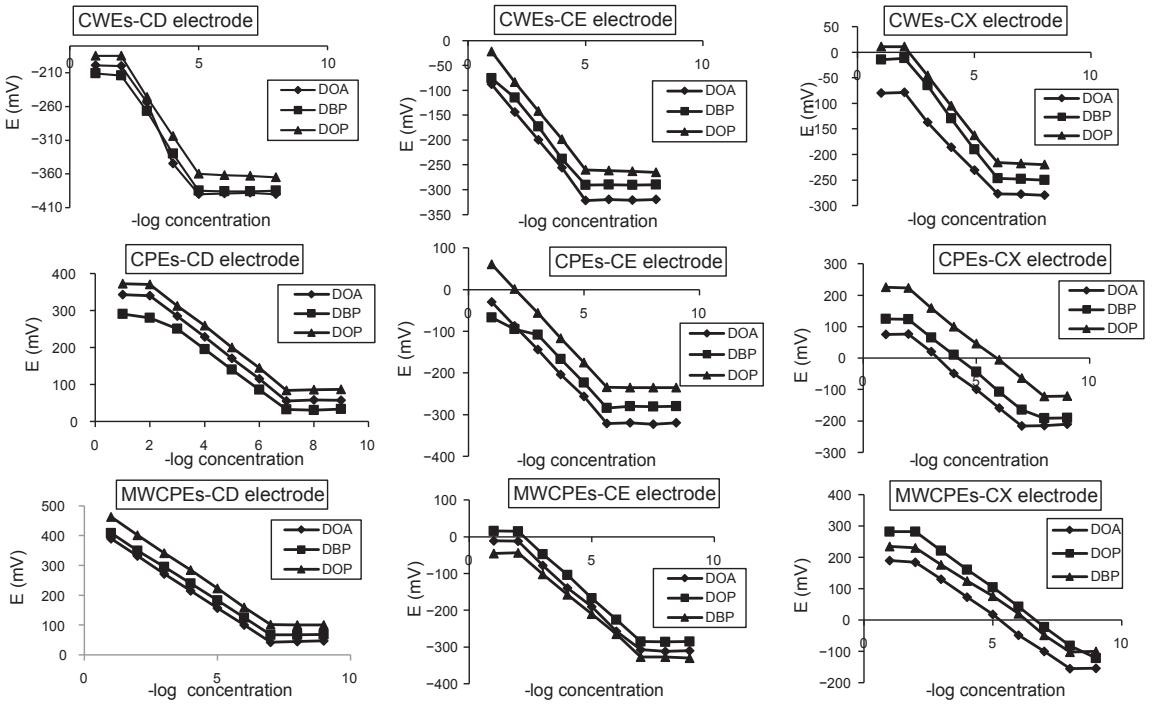


Fig. 2. Calibration graphs for the proposed CWEs, CPEs, and MWCPEs using three different plasticizers: DOP, DOA, and DBP.

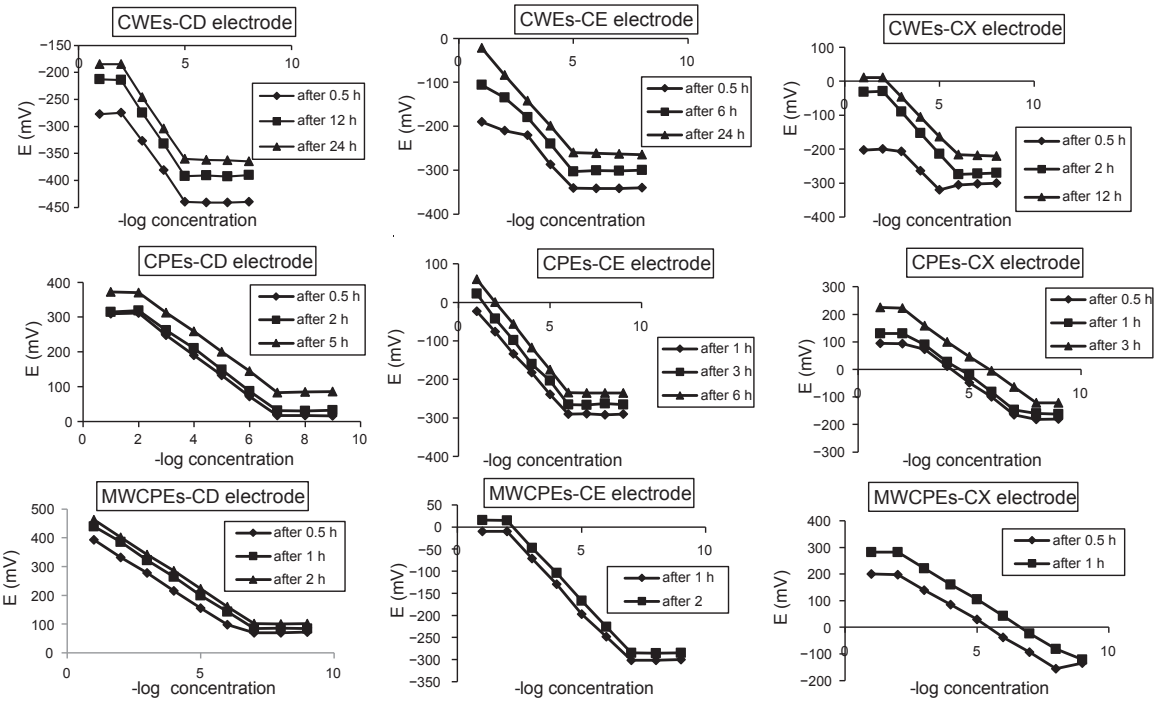


Fig. 3. Calibration graphs for the proposed CWEs, CPEs, and MWCPEs after soaking for different time intervals.

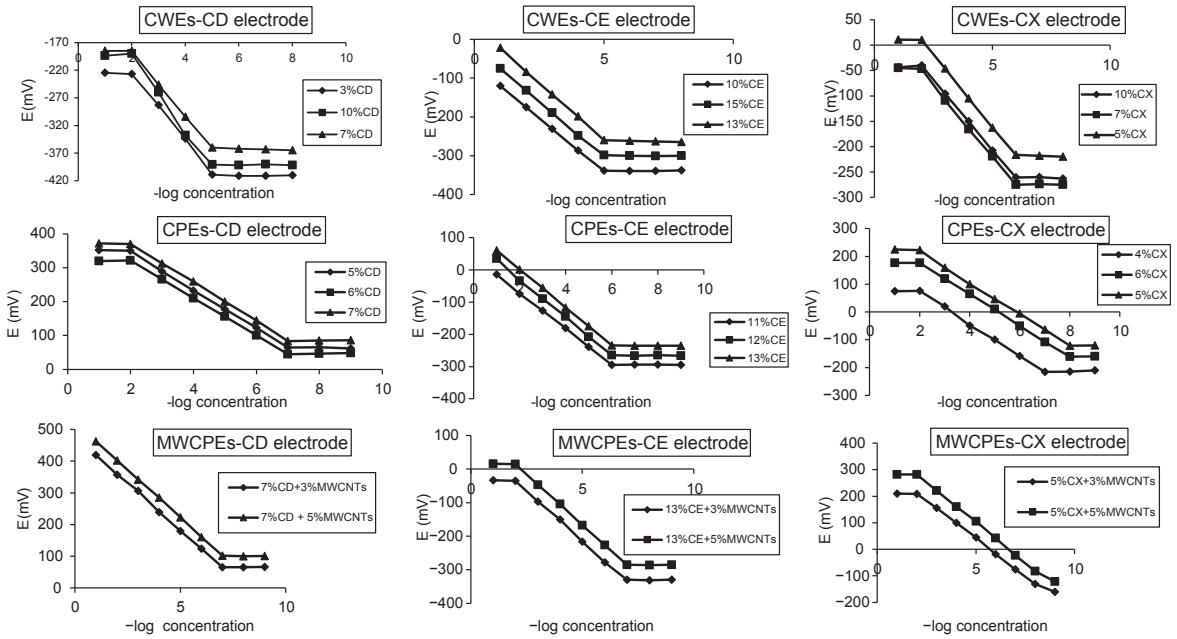


Fig. 4. Calibration graphs for the proposed CWEs, CPEs, and MWCPEs using different percentages of ionophores.

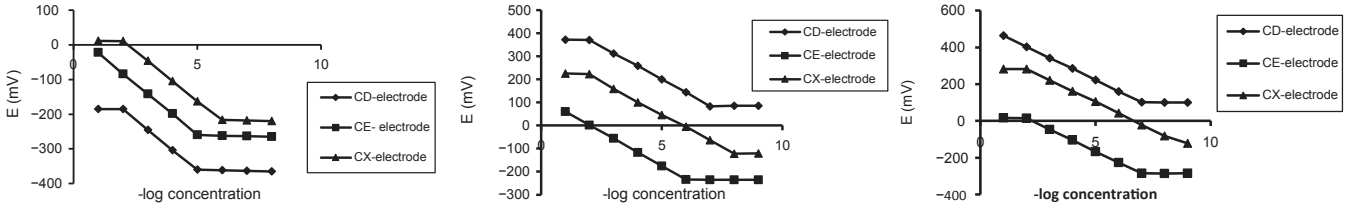


Fig. 5. Potentiometric calibration profile for tramadol hydrochloride in mol L^{-1} for (a) CWEs, (b) CPEs, and (c) MWCPEs.

3.3 Effect of soaking time on electrode performance

The effect of soaking of the nine proposed electrodes in 1×10^{-2} mol L^{-1} TR.HCl solution for different intervals was studied by measuring the corresponding slopes. For CWE-CD, CWE-CE, and CWE-CX electrodes, the slopes attained their maximum values of 56.36, 55.32, and 54.33 (mV/concentration decade) after soaking for 24, 24, and 12 h, respectively. The slopes started to decrease gradually to values of 49.37, 47.67, and 48.38 (mV/concentration decade) after soaking for 22, 33, and 30 d, respectively. For CPE-CD, CPE-CE, and CPE-CX electrodes, the optimum values of their slopes were achieved and recorded as 58.05, 57.66, and 56.08 (mV/concentration decade) after continuous soaking for 5, 6, and 3 h, respectively. They started to decrease, reaching values of

48.28, 49.11, and 50.10 (mV/concentration decade), after a period of 51, 50, and 60 d, respectively. For MWCPE-CD, MWCPE-CE, and MWCPE-CX, their maximum slopes of 60.53, 59.77, and 60.60 (mV/concentration decade) were reached after soaking for 2, 2, and 1 h, respectively. Then the slope values gradually decreased to 50.29, 49.37, and 50.54 (mV/concentration decade) after soaking for 78, 76, and 80 d, respectively. The effect of soaking time and its consequent effect on the response time and the effective concentration range were studied and are reported in Table 3. The electrodes' behavior after soaking for different time intervals is graphically represented in Fig. 3. On comparing the performances of the CWEs, CPEs, and MWCPEs, it is clear that MWCPEs require shorter time to attain Nernestian responses over a wide concentration ranges with higher slope values. This is mainly because of the nanostructure of CNT that tends to have a non-faradaic transduction mechanisms and accordingly, faster equilibrium is attained. Also the hydrophobic nature hinders the accumulation of water particles on the surface which facilitates the diffusion of TR.HCl particles through the electrode surface. The electrochemical performance characteristics of the proposed sensors are represented in Table 4.

3.4 Performance characteristics of electrodes

3.4.1 Dynamic response time

During the study the performance characteristics of the sensors, it was found that the time required for the electrodes to reach a stable potential reading after increasing the concentration 10-fold was remarkably minimized by using carbon nanotube modified carbon paste electrodes compared with using coated wire or carbon paste electrodes and reached ≈4 to 3 s. This proves that the incorporation of MWCNT to the membrane composition improves the performance of the electrodes by increasing the electrode conductivity. This is most probably due to the fast exchange kinetics of the association–dissociation of TR.HCl with the ionophores at the solution–membrane interface. The electrode potentials remained unaffected when measuring the concentrations of TR.HCl from low to high and from high to low, as represented graphically in Fig. 6.

3.4.2 Life-span of electrodes

The electrode life-span is the period over which the electrode optimally functions untill at least one of the performance characteristics deviates from its ideal value. The time spent by the proposed electrodes, starting from first soaking in the drug solution after the membrane fabrication to deviation from the ideal slope value by 95%, was measured and is shown in Table 4. The carbon nanotube modified carbon paste electrodes showed a longer life-span that reached more than 8 weeks with better response characteristics relative to other studied electrodes. The CWE-CD, CWE-CE, and CWE-CX electrodes life-span was 20, 23, and 25 d, respectively. For CPE-CD, CPE-CE, and CPE-CX electrodes, they were 46, 40, and 53 d, respectively. For MWCPE-CD, MWCPE-CE, and MWCPE-CX electrodes, they were 65, 60, and 72 d, which is of a great advantage especially in routine work in quality control laboratories. Regardless of the soaking time, a longer life-span of carbon paste electrodes relative to the coated wire ones was observed, as a renewable surface can easily be attained by squeezing out a small part of the paste and polishing it with filter paper to obtain a new surface. This allows the use of the electrodes for several months.

Table 3
Effect of soaking time on the performance of the proposed electrodes at 25 ± 1 °C.

Soaking time	Slope (mV/decade)	Usable concentration range (mol L^{-1})	Response time (s)	Soaking time	Slope (mV/decade)	Usable concentration range (mol L^{-1})	Response time (s)	Soaking time	Slope (mV/decade)	Usable concentration range (mol L^{-1})	Response time (s)
CWEs-β-CD electrode				CWEs-CE electrode				CWEs-CX electrode			
0.5 h	51.22	1.0×10^{-4}–1.0×10^{-2}	25	0.5 h	49.89	1.0×10^{-4}–1.0×10^{-1}	30	0.5 h	52.88	1.0×10^{-5}–1.0×10^{-2}	28
1 h	51.89	1.0×10^{-4}–1.0×10^{-2}	21	1 h	50.22	1.0×10^{-4}–1.0×10^{-1}	28	2 h	53.23	1.0×10^{-6}–1.0×10^{-2}	25
2 h	50.77	1.0×10^{-4}–1.0×10^{-2}	17	6 h	50.94	1.0×10^{-5}–1.0×10^{-1}	25	12 h	**54.33**	**1.0×10^{-6}–1.0×10^{-2}**	**20**
6 h	53.14	1.0×10^{-4}–1.0×10^{-2}	17	24 h	**55.32**	**1.0×10^{-5}–1.0×10^{-1}**	**17**	24 h	54.01	1.0×10^{-6}–1.0×10^{-2}	21
12 h	54.61	1.0×10^{-5}–1.0×10^{-2}	17	2 d	54.43	1.0×10^{-5}–1.0×10^{-1}	18	3 d	54.11	1.0×10^{-6}–1.0×10^{-2}	25
24 h	**56.36**	**1.0×10^{-5}–1.0×10^{-2}**	**15**	7 d	53.96	1.0×10^{-5}–1.0×10^{-1}	20	10 d	53.91	1.0×10^{-6}–1.0×10^{-2}	25
2 d	56.12	1.0×10^{-5}–1.0×10^{-2}	15	10 d	53.16	1.0×10^{-5}–1.0×10^{-1}	23	15 d	53.22	1.0×10^{-6}–1.0×10^{-2}	26
7 d	55.09	1.0×10^{-5}–1.0×10^{-2}	20	12 d	53.14	1.0×10^{-5}–1.0×10^{-1}	28	16 d	52.46	1.0×10^{-6}–1.0×10^{-2}	26
18 d	54.14	1.0×10^{-5}–1.0×10^{-2}	20	23 d	52.33	1.0×10^{-4}–1.0×10^{-1}	35	25 d	51.29	1.0×10^{-5}–1.0×10^{-2}	30
22 d	49.37	1.0×10^{-5}–1.0×10^{-2}	20	33 d	47.67	1.0×10^{-4}–1.0×10^{-1}	35	30 d	48.38	1.0×10^{-5}–1.0×10^{-2}	35
CPEs-β-CD electrode				CPEs-CE electrode				CPEs-CX electrode			
0.5 h	54.05	1.0×10^{-6}–1.0×10^{-2}	15	1 h	55.26	1.0×10^{-5}–1.0×10^{-1}	20	0.5 h	55.35	1.0×10^{-8}–1.0×10^{-2}	20
2 h	54.17	1.0×10^{-7}–1.0×10^{-2}	13	3 h	56.32	1.0×10^{-6}–1.0×10^{-1}	16	1 h	55.93	1.0×10^{-8}–1.0×10^{-2}	15
5 h	**58.05**	**1.0×10^{-7}–1.0×10^{-2}**	**9**	6 h	**57.66**	**1.0×10^{-6}–1.0×10^{-1}**	**10**	3 h	**56.08**	**1.0×10^{-8}–1.0×10^{-2}**	**15**
10 h	57.78	1.0×10^{-7}–1.0×10^{-2}	10	24 h	57.24	1.0×10^{-6}–1.0×10^{-1}	10	12 h	56.05	1.0×10^{-8}–1.0×10^{-2}	15
1 d	57.15	1.0×10^{-7}–1.0×10^{-2}	10	3 d	57.82	1.0×10^{-6}–1.0×10^{-1}	10	24 h	56.01	1.0×10^{-8}–1.0×10^{-2}	15
5 d	57.22	1.0×10^{-7}–1.0×10^{-2}	12	10 d	56.21	1.0×10^{-6}–1.0×10^{-1}	10	3 d	55.92	1.0×10^{-8}–1.0×10^{-2}	15
15 d	56.65	1.0×10^{-7}–1.0×10^{-2}	15	20 d	56.19	1.0×10^{-6}–1.0×10^{-1}	10	10 d	55.91	1.0×10^{-8}–1.0×10^{-2}	15
30 d	56.11	1.0×10^{-7}–1.0×10^{-2}	15	31 d	55.81	1.0×10^{-6}–1.0×10^{-1}	14	30 d	55.84	1.0×10^{-8}–1.0×10^{-2}	15
45 d	55.98	1.0×10^{-6}–1.0×10^{-2}	17	42 d	53.19	1.0×10^{-6}–1.0×10^{-1}	18	52 d	54.32	1.0×10^{-8}–1.0×10^{-2}	18
51 d	48.28	1.0×10^{-6}–1.0×10^{-2}	17	50 d	49.11	1.0×10^{-6}–1.0×10^{-1}	18	60 d	50.10	1.0×10^{-8}–1.0×10^{-2}	18
MWCPEs-β-CD electrode				MWCPEs-CE electrode				MWCPEs-CX electrode			
0.5 h	56.22	1.0×10^{-7}–1.0×10^{-1}	5	1 h	58.44	1.0×10^{-7}–1.0×10^{-2}	4	0.5 h	59.02	1.0×10^{-8}–1.0×10^{-2}	4
1 h	59.34	1.0×10^{-7}–1.0×10^{-1}	5	2 h	**59.77**	**1.0×10^{-7}–1.0×10^{-2}**	**3**	1 h	**60.60**	**1.0×10^{-8}–1.0×10^{-2}**	**4**
2 h	**60.53**	**1.0×10^{-7}–1.0×10^{-1}**	**3**	5 h	59.41	1.0×10^{-7}–1.0×10^{-2}	3	2 h	60.45	1.0×10^{-8}–1.0×10^{-2}	4
10 h	60.23	1.0×10^{-7}–1.0×10^{-1}	3	24 h	59.40	1.0×10^{-7}–1.0×10^{-2}	3	12 h	60.33	1.0×10^{-8}–1.0×10^{-2}	4
24 h	60.17	1.0×10^{-7}–1.0×10^{-1}	4	2 d	58.72	1.0×10^{-7}–1.0×10^{-2}	3	24 h	60.21	1.0×10^{-8}–1.0×10^{-2}	5
3 d	59.77	1.0×10^{-7}–1.0×10^{-1}	3	7 d	57.55	1.0×10^{-7}–1.0×10^{-2}	5	3 d	59.83	1.0×10^{-8}–1.0×10^{-2}	5
10 d	59.08	1.0×10^{-7}–1.0×10^{-1}	3	18 d	57.21	1.0×10^{-7}–1.0×10q	5	15 d	59.23	1.0×10^{-8}–1.0×10^{-2}	5
36 d	58.33	1.0×10^{-7}–1.0×10^{-1}	5	26 d	57.05	1.0×10^{-7}–1.0×10^{-2}	5	27 d	58.39	1.0×10^{-8}–1.0×10^{-2}	8
62 d	58.29	1.0×10^{-7}–1.0×10^{-1}	5	50 d	56.82	1.0×10^{-6}–1.0×10^{-2}	7	65 d	58.11	1.0×10^{-8}–1.0×10^{-2}	8
78 d	56.29	1.0×10^{-6}–1.0×10^{-1}	5	76 d	49.37	1.0×10^{-6}–1.0×10^{-2}	7	80 d	50.54	1.0×10^{-8}–1.0×10^{-2}	8

Table 4
Electrochemical performance characteristics of the TR.HCl sensors.

	CWEs			CPEs			MWCPEs		
	β-CD electrode	CE electrode	CX electrode	β-CD electrode	CE electrode	CX electrode	β-CD electrode	CE electrode	CX electrode
Slope (mV/decade)[a]	56.36	55.32	54.33	58.05	57.66	56.08	60.53	59.77	60.60
LOD (mol L^{-1})[b]	3.6×10^{-6}	2×10^{-7}	1×10^{-7}	1×10^{-8}	1×10^{-7}	4.9×10^{-9}	1×10^{-8}	1×10^{-8}	1×10^{-9}
Response time (s)	15	17	20	9	10	15	3	3	4
Working pH range	3–6	3–6	3–6.5	4–6.5	3–7	3.5–7	4–7	4.5–7	3–7.5
Concentration range (mol L^{-1})	1×10^{-5}–1×10^{-2}	1×10^{-5}–1×10^{-1}	1×10^{-6}–1×10^{-2}	1×10^{-7}–1×10^{-2}	1×10^{-6}–1×10^{-1}	1×10^{-8}–1×10^{-2}	1×10^{-7}–1×10^{-1}	1×10^{-7}–1×10^{-2}	1×10^{-8}–1×10^{-2}
Stability (d)	20	23	25	46	40	53	65	60	72
Average recovery% ± SD[a]	99.31 ± 0.62	99.36 ± 0.73	99.28 ± 0.67	99.8 ± 0.68	98.67 ± 0.67	99.56 ± 0.99	99.58 ± 0.93	99.03 ± 0.62	99.91 ± 0.31
Correlation coefficient	0.998	0.9992	0.9996	0.9995	0.9997	0.9997	0.9997	0.9998	0.9998
Repeatability (SDr)	0.78	0.68	0.63	0.67	0.53	0.48	0.59	0.47	0.45
Intermediate precision	1.15	1.36	1.28	1.09	0.96	0.85	0.91	0.88	0.65
Ruggedness[c]	98.76 ± 0.56	97.84 ± 0.83	98.65 ± 0.74	99.71 ± 0.58	97.86 ± 0.81	98.64 ± 0.87	99.16 ± 0.50	98.73 ± 0.76	99.57 ± 0.61

[a]Average of five determinations.
[b]Limit of detection (measured by intersection of the extrapolated arms of the calibration profile in Fig. 3 for each electrode).
[c]Average recovery percent of 10^{-5}, 10^{-4}, and 10^{-3} M for the proposed sensors using Mettler Toledo MP225 digital ion analyzer instead of Jenway 3510 digital ion analyzer.

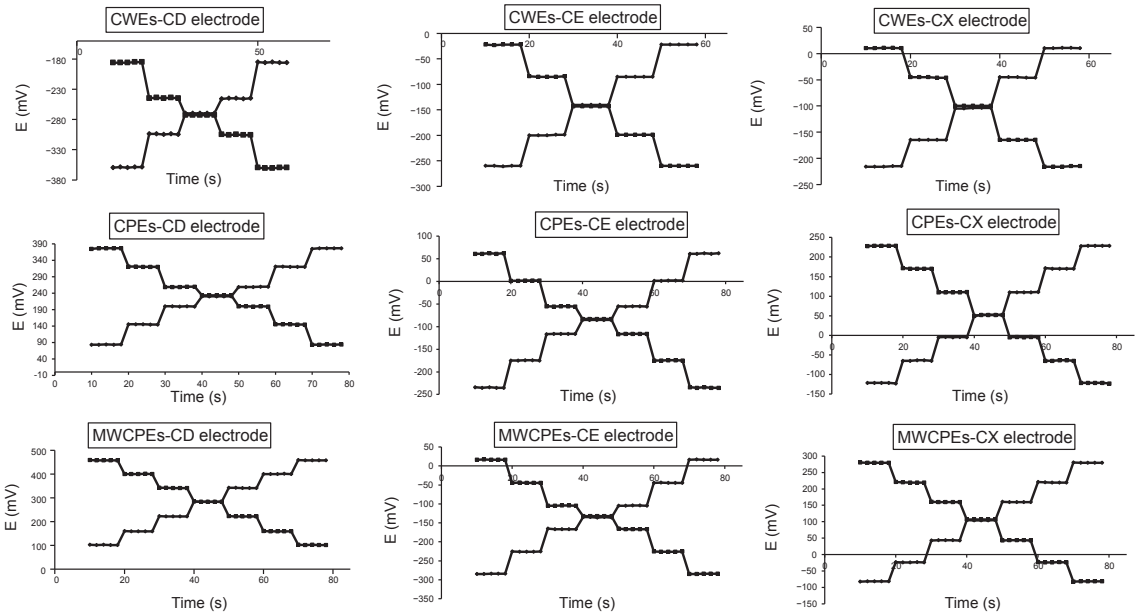


Fig. 6. Dynamic response time of the proposed CWEs, CPEs, and MWCPEs due to changing the tramadol concentration from low to high and from high to low.

3.4.3 Effect of pH and temperature

The effect of pH on the response of the proposed sensors was studied over the pH range of 2–10. As shown in Fig. 7, the TR.HCl potentials of all electrodes were almost constant over the pH range of 3–7. Therefore, these ranges can be used as the working pH ranges for the electrode assemblies. Moreover, it was noted that above pH 7, non-Nernestian slopes were observed, which can be attributed to the formation of the free tramadol base ($pK_a = 9.41$) in the test solution.

Upon studying the effect of temperature, it was found that the coated wire electrode potentials increased slightly with increasing temperature with minimal thermal stability up to 30 °C in comparison with carbon paste electrodes which showed thermal stability up to 50 °C without significant change in performance. However, the incorporation of carbon nanotubes increased the thermal stability of MWCPEs up to 80 °C. The calibration graphs obtained at different temperatures were parallel, and the limit of detection, slope, and response time did not significantly vary by increasing the temperature up to 80 °C.

3.4.4 Electrode selectivity coefficients

The potentiometric selectivities of the electrodes were measured relative to other ions which might be present in combination with TR.HCl, using the separate solution method and the matched potential method. The results presented in Table 5 reveal the high selectivity of all the electrodes to TR.HCL in the presence of inorganic cations, amino acids, sugars, and other co-administered pharmaceutical drugs, *e.g.*, paracetamol. This may be attributed to the difference in ionic size, mobility or permeability of the interfering ions to the membrane as compared with TR.HCl.

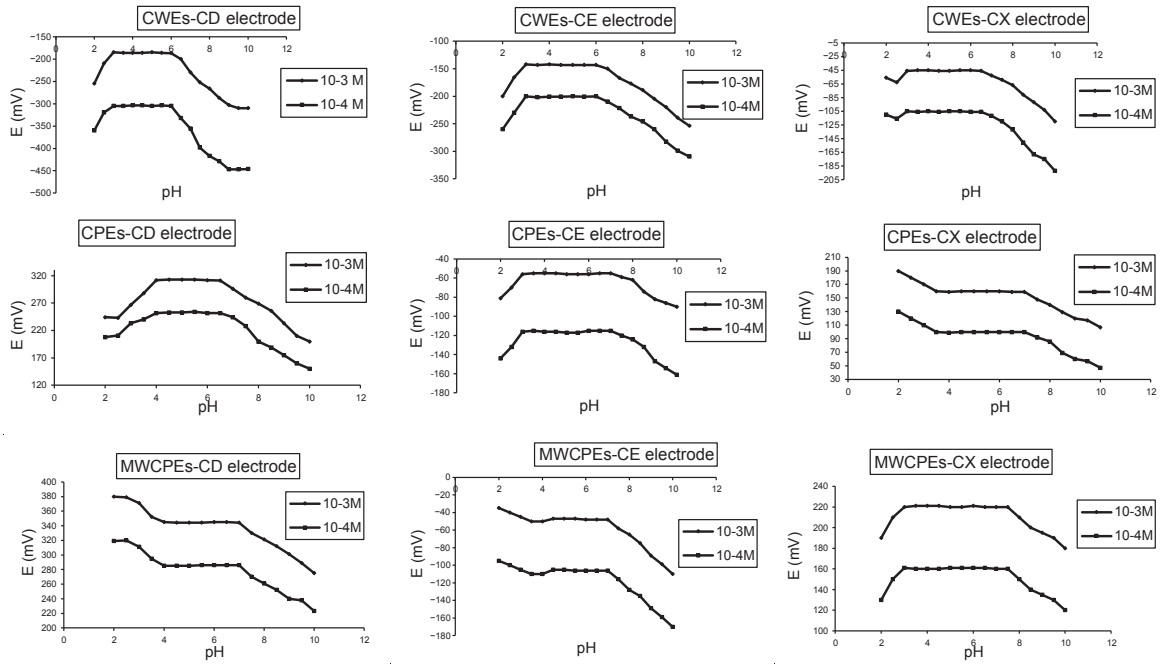


Fig. 7. Effect of pH on the response characteristics of the proposed CWEs, CPEs, and MWCPEs using two different concentrations for each electrode.

Table 5
Selectivity coefficients and tolerance values for TR-electrodes.

Interferent	$-\text{Log } k^{pot}_{drug,J}{}^{z+}$																	
	CWEs						CPEs						MWCPEs					
	β-CD electrode		CE electrode		CX electrode		β-CD electrode		CE electrode		CX electrode		β-CD electrode		CE electrode		CX electrode	
	SSM[a]	MPM[b]	SSM	MPM	SSM	MPM	SSM	MPM	SSM	MPM	SSM	MPM	SSM	MPM	SSM	MPM	SSM	MPM
Na^+	4.34	6.25	2.34	4.54	2.18	4.02	3.45	5.44	4.13	5.96	2.87	4.76	2.23	4.17	1.87	3.45	1.76	3.44
NH_4^+	4.22	6.18	3.69	5.36	3.05	4.94	3.28	5.17	3.54	5.43	2.45	4.31	2.54	4.38	2.56	4.47	2.05	4.18
K^+	3.56	5.03	3.27	5.34	2.57	4.32	2.48	3.54	2.67	4.60	2.09	4.11	2.61	4.53	2.83	4.72	2.19	4.28
Mg^{2+}	3.44	5.44	3.16	5.04	3.31	5.22	2.63	4.55	2.84	4.76	2.76	4.75	3.05	5.17	3.72	5.34	2.72	4.59
Ca^{2+}	4.17	6.08	4.66	6.47	2.81	4.78	2.71	4.61	2.36	4.27	3.43	5.67	2.68	4.98	3.56	5.57	3.90	6.02
Ba^{2+}	3.83	5.71	3.93	5.73	3.04	5.06	3.73	6.04	3.47	5.28	3.87	6.10	2.95	4.76	2.50	4.48	1.43	3.54
Cu^{2+}	4.15	6.03	2.56	4.47	4.26	6.18	4.01	6.06	3.81	5.79	4.07	6.45	2.32	4.56	3.41	5.39	1.32	3.31
Al^{3+}	4.28	6.16	2.54	4.42	2.64	4.53	3.55	5.42	2.44	4.37	3.16	5.38	3.11	5.17	3.25	5.18	2.46	4.46
Li^+	4.61	5.17	2.96	4.82	2.48	5.38	3.27	5.17	2.72	4.87	2.54	4.76	2.68	4.54	1.80	3.91	2.52	4.88
Fe^{2+}	3.25	4.73	3.48	5.25	3.27	5.19	3.62	5.46	3.42	5.45	3.63	5.67	3.54	5.42	3.05	5.13	2.11	4.26
L-Alanine	3.55	4.44	3.14	5.01	2.38	4.29	3.69	5.45	2.26	4.17	3.41	5.34	3.37	5.44	3.45	5.28	2.75	4.65
Glucose	3.05	4.28	4.08	5.99	3.35	5.18	2.43	4.28	3.15	5.23	2.52	4.38	3.80	5.74	2.93	5.06	2.64	4.55
Lactose	3.57	4.33	3.31	5.32	4.04	5.98	2.06	4.22	3.34	5.17	2.68	4.77	2.50	4.33	3.55	5.41	1.65	3.83
Propylene glycol	3.72	5.01	2.56	4.48	3.26	5.09	2.81	4.81	2.74	4.62	4.17	6.03	2.47	4.38	2.74	4.66	1.94	4.07
Paracetamol	4.55	5.45	2.45	4.51	2.72	4.54	3.28	5.33	2.26	4.32	3.44	5.27	3.27	5.43	2.59	4.34	1.54	3.56
Aspirin	4.36	5.24	3.34	5.19	2.46	4.23	3.14	5.31	3.76	5.44	2.95	4.90	3.18	5.07	2.87	4.29	2.44	4.76

[a]SSM: separate solution method.

[b]MPM: matched potential method.

3.5 Analytical applications of the studied electrodes

3.5.1 Potentiometric determination of TR.HCl in pharmaceutical formulation

The proposed sensors were applied for the analysis of TR.HCl in pharmaceutical dosage form Zaldiar® (37.5 mg TR.HCl). The results shown in Table 6 prove the applicability of the methods, as demonstrated by the excellent and precise percentage recoveries of TR.HCl. Analysis was carried out without prior treatment or extraction.

Statistical analysis of the results was applied using the t-test and F-test and showed no statistically significant differences between the results of the proposed methods and those obtained from the reported method in BP in the determination of TR.HCl in pharmaceutical tablets, which is based on non-aqueous titration with perchloric acid and potentiometrically determined end-point.

3.5.2 Potentiometric determination of TR.HCl in spiked human plasma and urine

The results presented in Table 7 prove the applicability of the proposed sensors to the determination of TR.HCl in spiked human plasma and urine over a wide concentration range of the drug that reached the nanogram level with high precision and accuracy. It is concluded that the proposed sensors can be successfully applied to in vitro studies and for clinical use.

3.5.3 Dissolution test

One tablet of Zaldiar® containing 37.5 mg TR.HCl was added to the dissolution medium of 900 ml 0.1N HCl and maintained at 37 ± 0.5 °C at 50 rpm for 45 min.[37] The potential reading corresponding to the amount of TR.HCl released at different time intervals was measured using the three CNT-modified electrodes. Figure 8 shows the release profile of TR.HCl at different time intervals. The results meet the requirement of TR.HCl dissolution that is not less than 70% of the drug is dissolved within 30 min.

3.6 Effect of water layer

Presence of a water layer between the ion selective membranes and the transducers may result in harmful effects since it can favor the presence of O_2 or CO_2 that diffuses through the membrane. The presence of O_2 can favor redox side-reactions, while CO_2 can change the pH of the interface, which may result in response drifts. Certain potential drifts where observed in CWEs responses when 1×10^{-3} mol L^{-1} ephedrine hydrochloride solution was replaced with 1×10^{-3} mol L^{-1} TR.HCl solution. This drift was not observed in case of MWCPEs as shown in Fig. 9. As the potentials of MWCPEs dropped rapidly into the negative direction and maintained a stable value and when removed from ephedrine hydrochloride solution, the potentials returned to their initial values. This means that no water layers were detected due to the highly hydrophobic character of the MWCNT inside the membranes.

Table 6
Determination of TR.HCl by the standard addition method using CWEs and statistical comparison of the data with the official method.

	CWEs								
	β-CD electrode			CE electrode			CX electrode		
	Taken (mol L^{-1})	Recovery	RSD	Taken (mol L^{-1})	Recovery	RSD	Taken (mol L^{-1})	Recovery	RSD
Pure solution	3×10^{-5}	98.91	0.71	5×10^{-5}	99.76	0.56	8×10^{-6}	98.92	0.47
	5×10^{-5}	99.32	0.34	9×10^{-5}	100.45	0.52	5×10^{-5}	99.56	0.62
	1×10^{-4}	99.55	0.46	1×10^{-4}	98.56	0.74	1×10^{-4}	100.34	0.57
	5×10^{-4}	100.30	0.59	5×10^{-3}	98.56	0.83	5×10^{-3}	99.29	0.49
	1×10^{-3}	98.47	0.34	1×10^{-2}	99.45	0.88	1×10^{-3}	98.31	0.44
Average ± SD	99.31 ± 0.62			99.36 ± 0.73			99.28 ± 0.67		
n	5			5			5		
Variance	0.38			0.53			0.45		
F-test (5.19)[a]	0.34			0.47			0.40		
Student t-test (2.262)[a]	0.66			0.53			0.70		
Zaldiar Tablet® (37.5 mg TR.HCl)	3×10^{-5}	99.56	0.85	5×10^{-5}	100.23	0.98	8×10^{-6}	98.76	0.45
	5×10^{-5}	100.18	0.93	9×10^{-5}	98.56	1.02	5×10^{-5}	97.58	0.38
	1×10^{-4}	98.55	0.65	1×10^{-4}	97.98	0.69	1×10^{-4}	99.83	0.76
	5×10^{-4}	98.72	0.61	5×10^{-3}	98.23	0.76	5×10^{-3}	99.52	0.44
	1×10^{-3}	100.64	0.73	1×10^{-2}	100.03	0.66	1×10^{-3}	98.67	0.37
Average ± SD	99.53 ± 0.81			99.00 ± 0.93			98.87 ± 0.79		
n	5			5			5		
Variance	0.66			0.84			0.62		
F-test (5.19)[a]	2.21			1.74			2.35		
Student t-test (2.262)[a]	0.78			1.56			1.88		
	CPEs								
	β-CD electrode			CE electrode			CX electrode		
	Taken (mol L^{-1})	Recovery	RSD	Taken (mol L^{-1})	Recovery	RSD	Taken (mol L^{-1})	Recovery	RSD
Pure solution	5×10^{-7}	100.76	0.55	5×10^{-6}	97.67	0.52	6×10^{-8}	100.18	0.34
	5×10^{-6}	100.23	0.48	3×10^{-5}	98.58	0.45	5×10^{-7}	101.03	0.51
	1×10^{-4}	98.76	0.37	5×10^{-4}	99.35	0.42	1×10^{-5}	99.52	0.33
	5×10^{-4}	99.50	0.62	1×10^{-3}	98.32	0.37	1×10^{-4}	98.13	0.40
	1×10^{-3}	99.75	0.49	5×10^{-2}	99.44	0.55	5×10^{-3}	98.96	0.52
Average ± SD	99.80 ± 0.68			98.67 ± 0.67			99.56 ± 0.99		
n	5			5			5		
Variance	0.46			0.45			0.98		
F-test (5.19)[a]	2.44			2.49			1.15		
Student t-test (2.262)[a]	0.28			1.86			0.15		
Zaldiar Tablet® (37.5 mg TR.HCl)	5×10^{-7}	98.07	0.54	5×10^{-6}	98.36	0.63	6×10^{-8}	100.34	0.56
	5×10^{-6}	99.65	0.32	3×10^{-5}	100.24	0.31	5×10^{-7}	98.83	0.48
	1×10^{-4}	99.17	0.58	5×10^{-4}	99.73	0.47	1×10^{-5}	98.47	0.61
	5×10^{-4}	99.93	0.51	1×10^{-3}	100.41	0.59	1×10^{-4}	99.51	0.33
	1×10^{-3}	99.26	0.38	5×10^{-2}	100.22	0.33	5×10^{-3}	97.56	0.41
Average ± SD	99.22 ± 0.71			99.79 ± 0.75			98.94 ± 0.94		
n	5			5			5		
Variance	0.51			0.56			0.88		
F-test (5.19)[a]	2.86			2.61			1.66		
Student t-test (2.262)[a]	1.35			0.37			1.65		
	MWCPEs								
	β-CD electrode			CE electrode			CX electrode		
	Taken (mol L^{-1})	Recovery	RSD	Taken (mol L^{-1})	Recovery	RSD	Taken (mol L^{-1})	Recovery	RSD
Pure solution	3×10^{-7}	99.17	0.36	5×10^{-6}	98.22	0.23	5×10^{-8}	100.12	0.27
	5×10^{-6}	98.26	0.28	5×10^{-5}	99.69	0.45	5×10^{-7}	100.03	0.38
	1×10^{-5}	100.11	0.33	5×10^{-4}	99.32	0.35	1×10^{-6}	99.36	0.34
	5×10^{-4}	101.02	0.41	5×10^{-3}	98.36	0.38	5×10^{-5}	100.22	0.41
	1×10^{-3}	99.36	0.25	1×10^{-3}	99.56	0.44	1×10^{-3}	98.81	0.43
Average ± SD	99.58 ± 0.93			99.03 ± 0.62			99.71 ± 0.61		
n	5			5			5		
Variance	0.86			0.38			0.37		
F-test (5.19)[a]	1.31			2.96			3.04		
Student t-test (2.262)[a]	0.12			1.21			0.21		
Zaldiar Tablet® (37.5 mg TR.HCl)	3×10^{-7}	100.47	0.21	5×10^{-6}	99.26	0.34	5×10^{-8}	99.27	0.43
	5×10^{-6}	101.21	0.23	5×10^{-5}	98.46	0.41	5×10^{-7}	99.90	0.35
	1×10^{-5}	100.65	0.33	5×10^{-4}	99.16	0.26	1×10^{-6}	98.26	0.32
	5×10^{-4}	99.67	0.43	5×10^{-3}	98.52	0.39	5×10^{-5}	98.55	0.27
	1×10^{-3}	99.72	0.35	1×10^{-3}	98.45	0.36	1×10^{-3}	99.27	0.28
Average ± SD	100.34 ± 0.58			99.00 ± 0.72			99.05 ± 0.58		
n	5			5			5		
Variance	0.34			0.52			0.34		
F-test (5.19)[a]	4.29			2.81			4.29		
Student t-test (2.262)[a]	0.59			1.71			1.72		

[a]The values in parentheses are the corresponding theoretical values of t and F at the 95% confidence level.

N.B.: The reported method average recovery ± SD is (99.65 ± 1.06), $n = 6$ for pure TR.HCl solution and is (100.01 ± 1.21), $n = 6$ for the pharmaceutical dosage form.

Table 7
Determination of TR.HCl in spiked human plasma and urine samples by the standard addition method.

	CWEs								
	β-CD electrode			CE electrode			CX electrode		
	Taken (mol L^{-1})	Recovery	RSD	Taken (mol L^{-1})	Recovery	RSD	Taken (mol L^{-1})	Recovery	RSD
	3×10^{-5}	98.65	0.78	5×10^{-5}	100.06	1.65	3×10^{-6}	98.56	0.94
	5×10^{-5}	97.58	0.85	8×10^{-5}	98.78	1.05	5×10^{-6}	99.54	0.83
Spiked human plasma	7×10^{-5}	98.14	0.76	1×10^{-4}	98.17	0.95	8×10^{-6}	97.72	1.01
	1×10^{-4}	99.05	0.94	4×10^{-4}	99.31	1.24	5×10^{-5}	97.41	0.87
	4×10^{-4}	97.69	1.05	2×10^{-4}	98.52	0.98	1×10^{-5}	98.22	0.93
Average ± SD	98.22 ± 0.63			98.97 ± 0.74			98.29 ± 0.83		
	3×10^{-5}	98.90	0.87	5×10^{-5}	100.14	1.02	3×10^{-6}	99.57	0.93
	5×10^{-5}	97.45	0.89	8×10^{-5}	98.67	0.78	5×10^{-6}	98.46	0.84
Spiked human urine	7×10^{-5}	98.44	1.75	1×10^{-4}	98.17	0.93	8×10^{-6}	98.07	0.81
	1×10^{-4}	96.35	1.65	4×10^{-4}	97.67	0.65	5×10^{-5}	97.56	0.65
	4×10^{-4}	99.02	1.43	2×10^{-4}	97.52	0.86	1×10^{-5}	97.05	0.76
Average ± SD	98.03 ± 1.13			98.43 ± 1.06			98.14 ± 0.96		
	CPEs								
	β-CD electrode			CE electrode			CX electrode		
	Taken (mol L^{-1})	Recovery	RSD	Taken (mol L^{-1})	Recovery	RSD	Taken (mol L^{-1})	Recovery	RSD
	8×10^{-7}	98.54	0.67	9×10^{-6}	100.23	0.82	8×10^{-8}	97.52	0.66
	6×10^{-7}	99.61	0.58	5×10^{-6}	98.48	0.93	5×10^{-8}	98.86	0.94
Spiked human plasma	7×10^{-6}	99.42	0.83	1×10^{-6}	98.17	0.77	5×10^{-7}	97.31	0.81
	4×10^{-6}	97.04	0.55	5×10^{-5}	97.24	0.73	1×10^{-7}	99.24	0.71
	1×10^{-5}	97.21	0.62	1×10^{-5}	97.35	0.84	5×10^{-6}	99.05	0.65
Average ± SD	98.36 ± 1.20			98.29 ± 1.20			98.40 ± 0.91		
	8×10^{-7}	99.56	1.23	9×10^{-6}	99.45	0.78	8×10^{-8}	99.36	0.67
	6×10^{-7}	100.12	1.41	5×10^{-6}	98.17	0.96	5×10^{-8}	97.76	0.79
Spiked human urine	7×10^{-6}	98.38	1.08	1×10^{-6}	97.42	1.04	5×10^{-7}	98.50	0.91
	4×10^{-6}	98.52	0.98	5×10^{-5}	97.81	1.22	1×10^{-7}	97.27	1.03
	1×10^{-5}	98.44	0.87	1×10^{-5}	99.83	1.35	5×10^{-6}	99.11	1.22
Average ± SD	99.00 ± 0.79			98.54 ± 1.05			98.40 ± 0.88		
	MWCPEs								
	β-CD electrode			CE electrode			CX electrode		
	Taken (mol L^{-1})	Recovery	RSD	Taken (mol L^{-1})	Recovery	RSD	Taken (mol L^{-1})	Recovery	RSD
	3×10^{-7}	100.07	1.24	5×10^{-6}	99.37	1.08	5×10^{-8}	100.23	0.87
	5×10^{-6}	98.87	0.97	5×10^{-5}	98.72	0.98	5×10^{-7}	99.89	0.93
Spiked human plasma	1×10^{-5}	97.47	0.86	5×10^{-4}	99.05	0.75	1×10^{-6}	98.71	0.76
	5×10^{-4}	98.69	0.82	5×10^{-3}	97.75	0.69	5×10^{-5}	98.54	0.72
	1×10^{-3}	99.43	0.67	1×10^{-3}	99.92	1.15	1×10^{-3}	99.15	0.88
Average ± SD	98.91 ± 0.97			98.96 ± 0.81			99.30 ± 0.74		
	3×10^{-7}	99.71	0.95	5×10^{-6}	98.46	1.32	5×10^{-8}	100.14	1.15
	5×10^{-6}	98.88	0.86	5×10^{-5}	99.24	0.87	5×10^{-7}	98.57	0.98
Spiked human urine	1×10^{-5}	97.61	0.89	5×10^{-4}	100.14	0.85	1×10^{-6}	99.07	0.89
	5×10^{-4}	99.08	1.09	5×10^{-3}	98.62	0.99	5×10^{-5}	97.54	0.93
	1×10^{-3}	100.34	1.13	1×10^{-3}	97.43	1.28	1×10^{-3}	97.89	0.78
Average ± SD	99.12 ± 1.02			98.78 ± 1.00			98.64 ± 1.03		

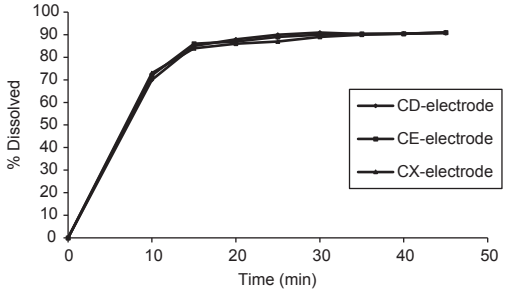


Fig. 8. Dissolution profile of Zaldiar® tablet (37.5 mg TR.HCl) using MWCPE-CE electrode and MWCPE-CX electrode.

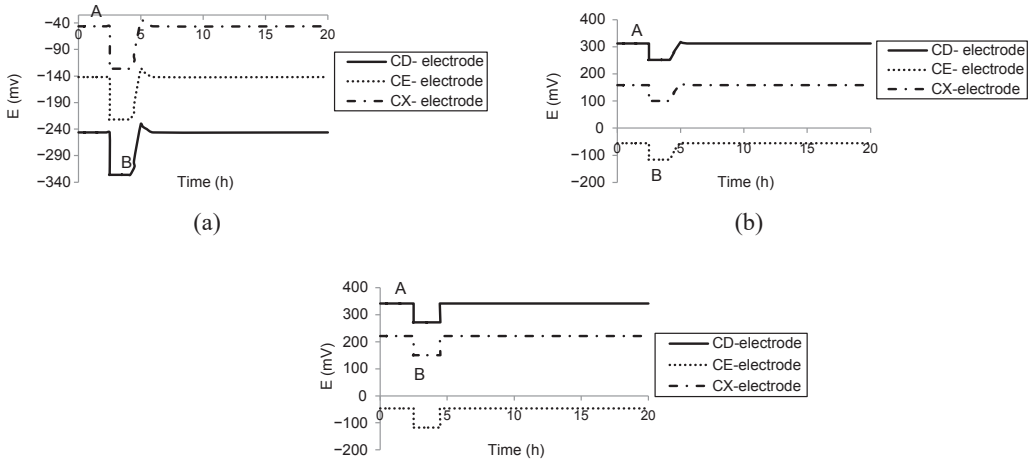


Fig. 9. Water layer test of TR.HCl selective electrodes. Area A: solution of 1×10^{-3} mol L^{-1} TR.HCl. Area B: solution of 1×10^{-3} mol L^{-1} ephedrine hydrochloride. (a) CWEs, (b) CPEs, and (c) MWCPEs.

4. Conclusion

The β-cyclodextrin, 18-crown-6-ether, and calix[4]arene ionophore-based coated wire, carbon paste, and CNT-modified carbon paste sensors offer successful techniques for TR.HCl determination. They are characterized as being highly stable, maintaining a linear Nernestian response for a period of over 2 months, and requiring a short conditioning time (6 h) to be used for quantitative analysis. They are also sufficiently accurate, sensitive, and selective for the quantitative determination of TR.HCl in pure form, pharmaceutical formulation and in spiked human plasma and urine. It was observed that the MWCPEs-CX sensor is sensitive, selective, and of long life time for TR.HCl determination. This may be attributed to the small molecular size of TR.HCl, which fitted the calix[4]arene cavity better than β-cyclodextrin or 18-crown-6-ether, which have larger inner diameters. The incorporation of MWCNTs adds a greater stability, sensitivity, and faster response to the electrodes. These can therefore be used for routine analysis of TR.HCl in quality control laboratories.

References

1 W. Leppert: Pharmacol. Reports **61** (2009) 978.
2 A. K. Rehni, I. Singh, and M. Kumar: Basic. Clin. Pharmacol. Toxicol. **103** (2008) 262.
3 H. Y. Ardakani and R. M. Rouini: Biopharm. Drug Dispos. **28** (2007) 526.
4 S. Grond and A. Sablotzki: Clin. Pharmacokinet. **43** (2004) 879.
5 A. S. Amin, A. M. Adawy, and E. A. Ali: Columban J. Life Sci. **17** (2015) 96.
6 O. A. Ismaiel and M. M. Hosny: Int. J. Instrum. Sci. **1** (2012) 34.
7 M. Sayed: J. Appl. Pharm. **6** (2014) 210.
8 K. N. Setty, T. Ramachar, I. E. Chakravarthy, and K. Prabhavathi: Chem. Sci. Trans. **1** (2012) 317.
9 D. Gharge and P. Dhabale: Int. J. Pharm. Technol. Res. **2** (2010) 1119.
10 G. Babu: Int. J. Appl. Pharm. Sci. Biomed. Sci. **2** (2013) 101.
11 A. A. Smith, R. Manavalan, K. Kannan, and N. Rajendiran: Int. J. Chem. Sci. **6** (2008) 789.

12 R. Shukla, R. Shivkumar, and K. N. Shivan: Bull. Pharm. Res. **1** (2011) 49.
13 P. Chandra, A. S. Rathore, S. Lohidasan, and K. R. Mahadik: Sci. Pharm. **80** (2012) 337.
14 T. Belal, T. Awad, and C. R. Clark: J. Chromatogr. Sci. **47** (2015) 849.
15 D. Ravisankar, V. Sridevi, and P. G. Kumari: Int. J. Appl. Biol. Pharm. Technol. **6** (2015) 174.
16 K. B. Ahir and E. M. Patelia: J. Chromatogr. Sep. Tech. **3** (2012) 1.
17 H. M. A. Shawish, A. R. Al-Dalou, N. A. Ghalwa, and A. A. A. Assi: Pharm. Anal. Acta **1** (2010) 1.
18 H. M. A. Shawish, N. A. Ghalwa, A. R. Al-Dalou, F. R. Zaggout, S. M. Saadeh, and A. A. A. Assi: Eurasian J. Anal. Chem. **6** (2011) 70.
19 M. R. Elghobashy, A. M. Mahmoud, M. R. Rezk, and M. K. Abd El-Rahman: J. Electrochem. Soc. **162** (2014) 1.
20 Y. Jingli, Y. Qiuhong, L. Donghui, and Z. Guizhi: Chin. J. Anal. Chem. **28** (2000) 84.
21 M. R. Ganjali, T. Razavi, F. Faridbod, S. Riahi, and P. Norouzi: Curr. Pharm. Anal. **5** (2009) 28.
22 M. M. Barsan, M. E. Ghica, and C. M. A. Brett: Anal. Chim. Acta **881** (2015) 1.
23 L. D. Chena and P. Buhlmann: Supramol. Chem. From Mol. Nanomater., eds. P. A. Gale and J. W. Steed (John Wiley & Sons Ltd., 2011) pp. 2539–2579.
24 K. Ariga and T. Kunitake: Supramolecular Chemistry—Fundamentals and Applications, 1st ed. (Springer-Verlag Heidelberg, Japan, 2006).
25 T. Ogoshi and A. Harada: Sensors **8** (2008) 4961.
26 P. Shahgaldian and U. Pieles: Sensors **6** (2006) 593.
27 Y. K. Agrawal, J. P. Pancholi, and J. M. Vyas: J. Sci. Ind. Res. (India) **68** (2009) 745.
28 Y. Umezawa, P. Bühlmann, K. Umezawa, K. Tohda, and S. Amemiya: Pure Appl. Chem. **72** (2000) 1851.
29 H. P. A. Nouws, C. Delerue-Matos, A. A. Barros, and J. A. Rodrigues: J. Pharm. Biomed. Anal. **42** (2006) 341.
30 E. Khaled, H. N. A. Hassan, G. G. Mohamed, and A. A. Seleim: Talanta **81** (2010) 510.
31 E. W. Baumann: Anal. Chim. Acta. **42** (1968) 127.
32 K. N. Mikhelson: Lecture Notes in Chemistry 81 Ion-Selective Electrodes, 1st ed. (Springer-Verlag Heidelberg, 2013).
33 F. Faridbod, M. R. Ganjali, R. Dinarvand, P. Norouzi, and S. Riahi: Sensors **8** (2008) 1645.
34 M. Feng, H. Han, J. Zhang, and H. Tachikawa: Electrochemical Sensors, Biosensors and their Biomedical Applications (Elsevier Inc., 2008) pp. 459–501.
35 M. P. Pujado: Carbon Nanotubes as Platforms for Biosensors with Electrochemical and Electronic Transduction, 1st ed. (Springer-Verlag Heidelberg, 2012).
36 R. N. Goyal, M. Oyama, V. K. Gupta, S. P. Singh, and R. A. Sharma: Sens. Actuators, B **134** (2008) 816.
37 USP Convention revision bulletin (2010), 1-3 available online at http://www.usp.org/usp-nf/official-text/accelerated-revision-process/accelerated-revision-history/acetaminophen-and-tramadol-hydrochloride-tablets.