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An electrochemical sensing platform to determine tetrahydrozoline HCl in pure form, pharmaceutical formulation, and rabbit aqueous humor†

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In the pharmaceutical industry, finding cost-effective and real-time analyzers that provide valid data is a good aim. The purpose of this work was to propose a link between the pharmaceutical industry and the recent innovations in solid-contact ion-selective electrodes (SC-ISEs) for the utilization of these electrodes as real-time analyzers to evaluate the concentration of tetrahydrozoline HCl in different matrices. The backbone of these new potentiometric sensors is the conjunction of calix[6]arene and (2-hydroxypropyl)-β-cyclodextrin as molecular recognition elements and a network of multi-walled carbon nanotubes as a solid transducer material between an ionophore-doped PVC membrane and microfabricated Cu electrodes. The proposed sensors were optimized to determine tetrahydrozoline, and their performances were assessed according to the IUPAC recommendations. The proposed solid-contact sensors were compared with liquid contact sensors, and the former sensors were found to be better than the latter sensors in terms of durability, handling, and easier adaptation to industry with comparable sensitivity. The measurements were implemented using phosphate buffer (pH: 6). The best obtained linearity range was 1×10^{-2} to 1×10^{-7} M, and the best LOD was 1×10^{-8} M. The sensors with the best performance were successfully applied to determine tetrahydrozoline in a pharmaceutical eye preparation and rabbit tears. The obtained results were statistically compared to those obtained by the official method of analysis, and no significant difference was obtained. The eco-score of the method was assessed using the eco-scale tool and also compared with that of the official method. The proposed approach was validated according to the International Council for Harmonisation (ICH) guidelines.

1. Introduction

Tetrahydrozoline (THZ) HCl (1*H*-imidazole, 4,5-dihydro-2-(1,2,3,4-tetrahydro-1-naphthalenyl), monohydrochloride) is a sympathomimetic agent used as a nasal decongestant due to its alpha-adrenergic effect, leading to vasoconstrictor activity, its structure is shown in Fig. 1. THZ is combined with antibiotics, corticosteroids, and anti-allergics to formulate anti-infective eye preparations for the treatment of acute and sub-acute conjunctivitis, keratitis, and corneal ulcers.[1] The detection of THZ at low concentrations in biological fluids is very useful to determine its overdose, especially if accidently ingested by children.[2] Different methods, such as spectroscopy,[3–8] TLC-densitometry,[9] HPLC,[10–13] gas chromatography,[14] and an electrochemical method,[15] have been reported for the determination of THZ in pharmaceutical dosage forms and biological solutions. After investigating the literature, we observed that to

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date, no solid electrochemical sensor has been developed to quantify THZ.

Potentiometry with ion-selective electrodes (ISEs) is a non-destructive technique that offers a lot of advantages such as portability, low energy consumption, short analysis time, and adaptability to small sample volumes. These advantages have expanded the application of ISEs in pharmaceutical analysis. Moreover, a very attractive feature of potentiometric ISEs is their ability to determine the analytes without sample preparation *via*
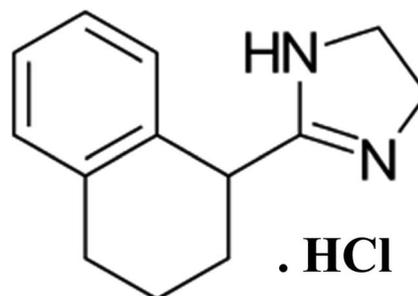

Fig. 1 Chemical structure of tetrahydrozoline HCl.

a compact and low-cost instrument.[16,17] Generally, electrochemical detection is also considered as one of the greenest methods of analysis.[18]

In potentiometric applications, enhancing selectivity and sensitivity is the main aim that guide the optimization during method development. A key component in ISE fabrication that can significantly improve the selectivity and sensitivity is doping the PVC membrane with an ionophore.[19] Ionophore is a macromolecule that can bind or form a complex with the target ion in an aqueous sample solution to help the transfer of the target ion form the aqueous sample solution to the lipophilic membrane by decreasing the Gibbs free energy of transfer and hence improving the sensitivity.[20] Supramolecular ionophores, such as (2-hydroxypropyl)-β-cyclodextrin (2-HP βCD) and calixarenes (CX), both of which have a three-dimensional basket, cup or bucket shape,[21] play an important role in electrochemical sensors. Their special structure with a lipophilic interior helps in selectively capturing the target ion.[22] They are utilized in ion-selective electrodes, selective membranes, and stationary phases[23] and have been extensively used in electrochemical sensing.[24–27] A synergistic effect between these ionophores and nanocomposites (carbon nanotubes) can help in the specific determination of THZ in the presence of other ions.

Nanoparticles have attracted the attention of researchers for the fabrication of optimized stable solid-contact ion-selective electrodes (SC-ISEs) for several applications.[28–31] Sensor components are vital factors that affect the ability of a sensor to determine the target drug molecule.[28] Carbon nanotubes (CNTs) have a unique structure with substantial electrical and thermal properties.[29] CNT nanocomposites are simply deposited over various surfaces; this makes them ideal for application in SC-ISEs. They have been utilized as an ion-to-electron transducer coating in ISEs[31,32] in addition to being integrated in the assembly of biosensors.[33,34] CNTs can reduce signal drift, which is a major disadvantage of solid-contact membranes.[35] CNTs have various advantages over polymers that are commonly used as transducers.[30] First, CNTs are hydrophobic; therefore, they practically prevent the formation of a water layer at the interface between the polymer membrane and the electrode. Second, CNTs are inert and do not undergo redox reactions; thus, they are stable under an O_2 and CO_2 atmosphere. Third, CNTs are not sensitive to light.

The objective of this research was the fabrication of SC-ISEs with optimum performance and improved sensitivity to quantify THZ in both a pharmaceutical formulation and aqueous humor. In order to accomplish the stepwise optimization of a cation exchanger, plasticizer, and ionophore, we performed an initial screening using the conventional liquid contact membrane, and then, the optimized sensing film composition was applied to Cu-microfabricated electrodes and screen-printed electrodes in combination with CNTs as a transducer layer. Moreover, we evaluated the greenness of the proposed analytical method and compared its performance and greenness to those of the official method for the assay of THZ.

2. Materials and methods

2.1. Chemicals and reagents

Pure tetrahydrozoline HCl was kindly supplied by Orchidia Pharmaceutical Company, Cairo, Egypt, and its purity was certified to be 99.91 ± 0.11%. Efemyo® eyedrops (batch number 1018201) by Orchidia pharmaceutical company (Egypt) was purchased from the local market. Efemyo® is labeled to contain 1 : 0.25 mg mL^{-1} fluorometholone (FLM) and THZ, respectively. All chemicals and solvents were of analytical grade. High-molecular-weight polyvinyl chloride (PVC), sodium tetrakis [3,5-bis(1,1,1,3,3,3-hexa-fluoro-2-methoxy-2-propyl)phenyl] borate trihydrate (TFPB), potassium tetrakis(4-chlorophenyl) borate (TCPB), sodium tetraphenylborate (NaTPB), 2-nitrophenyl octyl ether (*O*-NPOE), dibutyl sebacate (DBS), tetrahydrofuran (THF), calix[6]arene (CX6), (2-hydroxypropyl)-β-cyclodextrin (2-HP βCD), and multiwalled carbon nanotube powder (MWCNT) were obtained from Fluka (Steinheim, Germany). KCl, HCl, NaOH, and NaH$_2$PO$_4$ were obtained from Sigma-Aldrich (Steinheim, Germany). Carbon screen-printed electrodes (3 mm diameter) were purchased from CH Instruments (Austin, TX). Modified Cu microfabricated electrode was fabricated using a blend of photolithography and drop-casting techniques. Distilled deionized water was obtained from Alpha pure laboratories (Cairo, Egypt). Phosphate buffer with pH 6 was prepared according to the British Pharmacopoeia (BP).[36]

2.2. Instruments

Jenway digital potentiometer model 3510 (Essex, UK) with a Ag/AgCl double junction as a reference electrode was purchased from Aldrich Chemical Co., Steinheim, Germany. A glass pH-sensing electrode was obtained from Jenway, UK.

2.3. Standard solutions

2.3.1. Stock standard solution of THZ. Herein, a 1×10^{-2} M solution of THZ was prepared by transferring accurately weighed 0.237 g of THZ HCl to a 100 mL volumetric flask, dissolving it with buffer (pH: 6), and then completing the volume to the mark using the same buffer (pH: 6).

2.3.2. Working standard solutions of THZ. Different solutions of THZ having different strengths in the 1×10^{-8} to 1×10^{-3} M range were freshly prepared by sequentially diluting the stock solutions with phosphate buffer (pH: 6) in a series of 25 mL measuring flasks and then completing the volume to the mark using phosphate buffer (pH: 6).

2.3.3. Rabbit aqueous humor. Rabbit aqueous humor was purchased from the animal house of Cairo university.

2.4. Fabrication of sensors

2.4.1. Liquid contact membranes. In a set of 6 glass petri dishes (5 cm diameter), 0.4 mL of a plasticizer was thoroughly mixed with 190 mg of PVC and 10 mg of a cation exchanger (TCPB for the sensors I & II, TFPB for the sensors III & IV, and NaTPB for the sensors V & VI) separately. The mixtures were

Table 1 The composition of ion sensitive membranes utilized in optimizing liquid-conventianl ion-selective electrodes

Sensor	Polymer matrix	Plasticizer	Cation exchanger	Ionophore
I	PVC	NPOE	TCPB	—
II	PVC	DBS	TCPB	—
III	PVC	NPOE	TFPB	—
IV	PVC	DBS	TFPB	—
V	PVC	NPOE	NaTPB	—
VI	PVC	DBS	NaTPB	—
VII	PVC	NPOE	TFPB	CX6
VIII	PVC	NPOE	TFPB	2-HP βCD
IX	PVC	NPOE	TCPB	CX6
X	PVC	NPOE	TCPB	2-HP βCD

plasticized using different plasticizers to evaluate the best components and then dissolved in 6 mL of THF. The petri dishes were covered with filter papers and left overnight to allow the solvent to dry at room temperature. A master membrane with an approximately 0.1 mm thickness was obtained from each petri dish. The different plasticizers and ion-pairs used herein are presented in Table 1.

Moreover, two commercially available ionophores (CX6 and 2-HP βCD) were selected for selectivity screening and to improve the sensor performance. The same abovementioned procedures were conducted along with the addition of 10 mg of either CX6 or 2-HP βCD to the PVC matrix to obtain the sensors VII–X (Table 1).

2.4.2. Solid contact membranes. The optimum membrane matrix (TFPB/O-NPOE/CX6) of the prepared liquid contact membranes that provided the best performance was drop-casted on the surface of three solid contact sensors, namely, Cu-microfabricated electrode (Cu), carbon screen-printed electrode (C-SPE), and carbon screen-printed electrode with MWCNT (C-SPE/MWCNT),[37] to obtain the sensors XI–XIII, as shown in Fig. 2.

2.5. Electrode assembly

2.5.1. Liquid contact membranes. From the prepared liquid membrane, an approximately 8 mm diameter disk was removed using a cork borer and then connected using THF to a PVC tip that was attached to the end of the electrode body. The electrode was then loaded with equal volumes of 1×10^{-3} M THZ and 1×10^{-3} M KCl (prepared in phosphate buffer, pH 6)
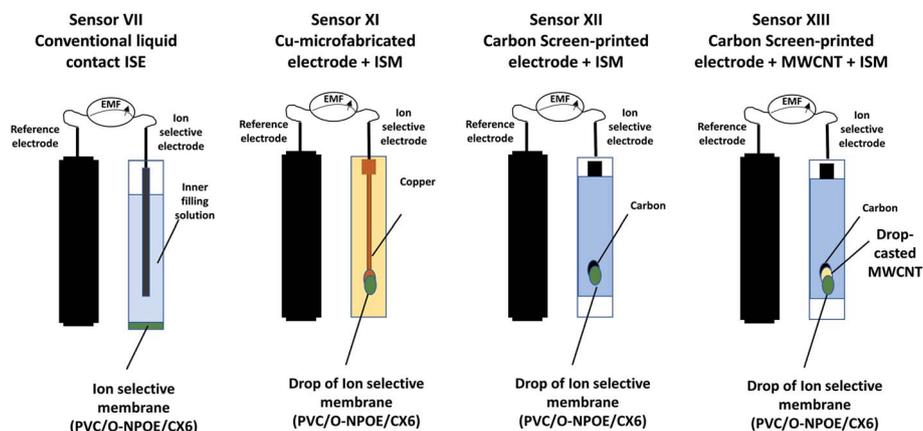

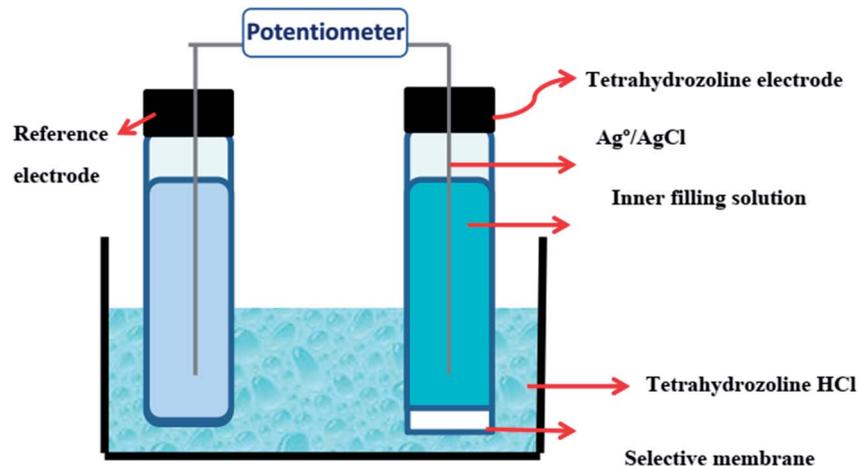**Fig. 2** Schematic representing the sensors VII and XI–XIII.

Fig. 3 A schematic of the potentiometric determination of tetrahydrozoline HCl.

as internal filling solutions. Subsequently, a Ag/AgCl wire with 1 mm diameter was immersed in this inner filling as an internal reference, as shown in Fig. 3. Preconditioning of the sensor before the first use was achieved by soaking the sensor in a 1×10^{-3} M THZ standard solution for 24 hours, and the sensor was stored using distilled water when not in use.

2.5.2 Solid contact membranes. The solid contact electrodes were fabricated by drop casting 5 μL of the sensing mixture TFPB/O-NPOE/CX6 on the surface of Cu and C-SPE to achieve the sensors XI & XII; however, for the sensor XIII, 5 μL of 1% MWCNT nanocomposite in THF, prepared following the same procedure as reported in the literature,[37] was applied before the application of the sensing solution.

2.6. Sensor calibration

The sensors in conjunction with the reference electrode were immersed in THZ standard solutions with concentrations in the range of 1×10^{-8} to 1×10^{-2} M that were prepared in the buffer solution (pH: 6). Conditioning was allowed under stirring until a stable reading of the potentiometer was attained. The electromotive force (*emf*) readings within ±1 were recorded at room temperature. The sensors were washed with the buffer (pH: 6) pre and post each run until a stable potential was achieved. Calibration curves were obtained by plotting the recorded electrode potential of the proposed sensors *versus* the −log molar concentrations of THZ. The regression equations for the linear part of the curves were computed, as presented in Table 2.

2.7. Effect of pH

The effect of pH on the potential values of the sensor was investigated using 1×10^{-3} M and 1×10^{-4} M solutions of THZ. Various pH values ranging from 2 to 10 were used, achieved by adding either 0.1 M HCl or 0.1 M NaOH. The obtained potential was recorded at each pH value, as shown in Fig. 4.

2.8. Sensor selectivity

2.8.1. Interfering ions. The potential reading of the sensors was recorded in the presence of related electrically active molecules. The degree of interference was estimated by the selectivity coefficient that was computed by the separate solution method (SSM)[38] using the following equation:

$$(K_{\text{Primary ion,interferent}}^{\text{Pot}}) = E_1 - E_2/S$$

where E_1 is the potential measured in 1×10^{-3} M of THZ in buffer (pH: 6), E_2 is the potential measured in 1×10^{-3} M of the interferent ion solution in the same buffer solution, and S is the slope of the calibration plot for the examined sensor, as presented in Table 3.

2.9. Application of the analytical procedures

2.9.1. Pharmaceutical formulations. The proposed procedure was applied to determine the concentration of THZ in the Efemyo® ophthalmic preparation. Accurately measured 2.4 mL of Efemyo® was transferred to a 25 mL volumetric flask. The volume was brought up to the mark using the buffer solution

Table 2 System suitability parameters of the developed sensors

Parameter	Sensor I	Sensor II	Sensor III	Sensor IV	Sensor V	Sensor VI	Sensor VII	Sensor VIII	Sensor IX	Sensor X	Sensor XI	Sensor XII	Sensor XIII
Validation of the regression equations:													
Slope[a] (mV per decade)	54.72	51.63	56.39	53.96	54.15	48.03	57.11	55.4	56.81	55.13	53.72	55.96	58.54
Intercept[a]	216.36	293.5	231.32	219.91	209.25	187.28	316.03	261.32	241.5	254.56	283.8	313.18	319.1
Correlation coefficient (r)	0.9997	0.9996	0.9996	0.9994	0.9994	0.9996	0.9993	0.9997	0.9997	0.9971	0.9993	0.9997	0.9994
Validation of the responses:													
Concentration range (M)	1×10^{-6} to 1×10^{-2}	1×10^{-6} to 1×10^{-2}	1×10^{-6} to 1×10^{-2}	1×10^{-6} to 1×10^{-2}	1×10^{-6} to 1×10^{-2}	1×10^{-6} to 1×10^{-2}	1×10^{-7} to 1×10^{-2}	1×10^{-6} to 1×10^{-2}	1×10^{-6} to 1×10^{-2}	1×10^{-6} to 1×10^{-2}	1×10^{-6} to 1×10^{-2}	1×10^{-6} to 1×10^{-2}	1×10^{-7} to 1×10^{-2}
Working pH range	2–8	2–8	2–8	2–8	2–8	2–8	2–8	2–8	2–8	2–8	2–8	2–8	2–8
Response time (s)	5	10	10	12	14	9	5	10	10	12	10	10	9
Stability (weeks)	4	4	4	4	4	3	4	4	4	4	6	7	10
Accuracy[b]	99.56	100.37	99.01	100.32	101.1	100.13	99.98	100.34	100.78	99.82	100.34	100.78	99.82
Repeatability[c]	0.258	1.174	0.309	0.214	0.4135	0.974	0.245	1.34	0.42	0.64	1.34	0.42	0.64
Intermediate precision[c]	0.908	1.527	1.361	0.98	1.235	1.525	0.886	1.48	1.2	0.98	1.48	1.2	0.98
LOD[d]	6.3×10^{-7}	7.9×10^{-7}	3.9×10^{-7}	8.9×10^{-7}	6.3×10^{-6}	7.9×10^{-6}	5.01×10^{-8}	3.9×10^{-7}	2×10^{-7}	5.1×10^{-7}	6.3×10^{-7}	3.16×10^{-7}	8.1×10^{-8}

[a] Average of three determinations. [b] Accuracy results were done for (5×10^{-5}, 5×10^{-4} and 5×10^{-3} M) of THZ. [c] Repeatability and intermediate precision results were done for (10^{-5}, 10^{-4} and 10^{-3} M) of THZ. [d] LOD (limit of detection) was measured by the interception of the extrapolated arms of the calibration curves.
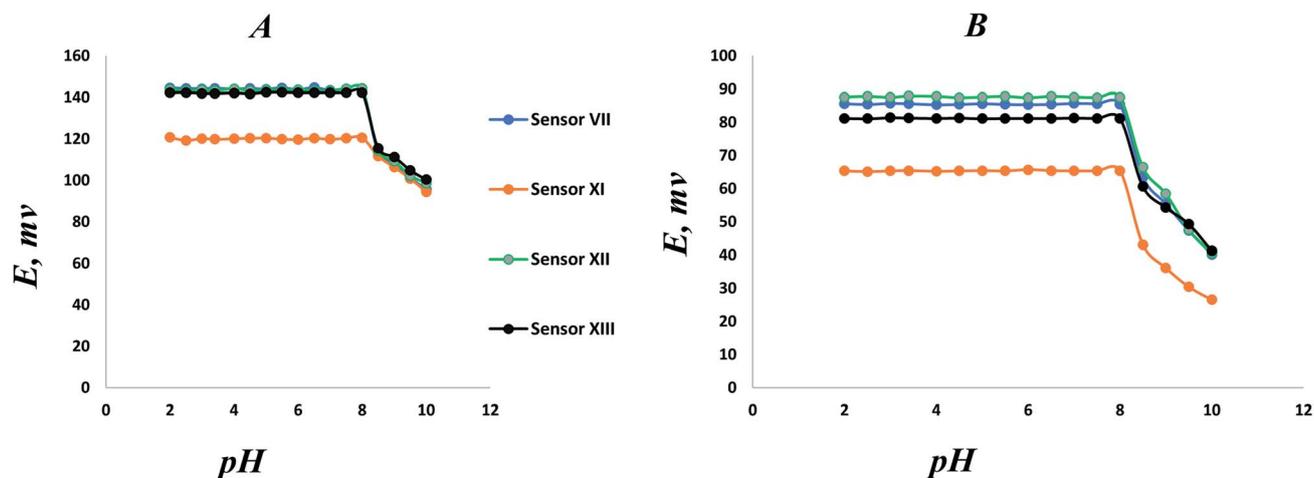
Fig. 4 The effect of pH on the developed sensors using a (A) 10^{-3} M TZH solution and (B) 10^{-4} M TZH solution.

(pH: 6). The concentration of THZ in this solution was found to be 1×10^{-3} M. The solutions were transferred to separate 25 mL beakers, and the potential was measured by immersing the prepared electrode in conjunction with the reference electrode in each solution. The concentration of THZ was computed from the regression equation, and the results are presented in Table 4.

2.9.2. Rabbit aqueous humor. Herein, 0.5 mL aliquot of the aqueous humor was taken in two separate 5 mL measuring flasks. The volume was brought up to the mark with 1×10^{-5} M and 1×10^{-6} M standard drug solutions, prepared in the buffer (pH: 6), respectively, and the two flasks were vortexed for 1 minute. The potentiometric measurements were performed using the proposed sensor, and the concentration of THZ was computed from the regression equation, as presented in Table 5.

3. Results and discussion

3.1. Membrane composition

The ISM consists of a polymer matrix (to provide mechanical support to the membrane), a plasticizer that dissolves the ion

association complex, plasticizes the membrane, and affects the lipophilicity of the polymer membrane, a hydrophobic ion (known as the ion exchanger) with a charge opposite to that of the target ion, and an ionophore that selectively binds to the target ion. The plasticizer also alters the distribution coefficient (K_d) of different species, thus affecting the performance characteristics of the electrode.[39] An electrical potential—known as the phase-boundary potential—is generated at the interface of the ISM and the sample solution by selective partitioning of the ionic species between these two phases, resulting in interfacial charge separation. The magnitude of the phase-boundary potential depends on the activity of the ion in the membrane and the aqueous solution. The modifiers calixarene and cyclodextrin used herein are ionophores. The use of an ionophore with a higher binding strength to that of THZ lowers the free energy of transfer of THZ from the aqueous phase to the ISM and results in a stronger signal of the ISE to THZ.[40]

SC-ISEs were initially prepared in the form of coated wire electrodes by directly coating the ion-selective membranes on metallic electrodes.[41] However, the ill-defined interface between

Table 3 Potentiometric selectivity coefficients ($K_{1ry\ ion}^{pot}$) of the four proposed sensors obtained using the separate solution method (SSM)

Interferents[a] (10^{-3} M)	Sensor VII	Sensor XI	Sensor XII	Sensor XIII
NaCl	−3.35	−3.83	−3.76	−3.62
KCl	−3.52	−3.53	−3.30	−3.13
CaCl$_2$	−3.18	−3.83	−3.59	−3.83
MgCl$_2$	−3.70	−3.61	−3.96	−3.91
Urea	−3.71	−3.43	−3.98	−3.86
Glucose	−3.52	−3.50	−3.76	−3.99
Hydroxy propyl methyl cellulose	−3.22	−3.84	−3.12	−3.16
Macrogel	−3.85	−3.62	−3.56	−3.91
Benzalkonium chloride	−3.82	−3.72	−3.96	−3.64
Ofloxacin	−3.58	−3.57	−3.90	−3.27
Gatifloxacin	−3.33	−3.36	−3.79	−3.10
Prednisolone acetate	−3.19	−3.63	−3.62	−3.32
Dexamethasone sodium phosphate	−3.47	−3.55	−3.12	−3.85
Chloramphenicol	−3.70	−3.66	−3.91	−3.88

[a] $K_{Primary\ ion,interferent}^{Pot} = E_1 - E_2/S$.

Table 4 Determination of THZ in Efemyo eyedrops by the suggested sensors

Pharmaceutical formulation	Sensors	Found%[a]	R.S.D%
Efemyo® eyedrops labelled to contain (1% FLM and 0.25% THZ) batch number 1018201	Sensor VII	99.29	1.006
	Sensor XI	99.77	1.057
	Sensor XII	99.97	1.183
	Sensor XIII	98.803	0.899

[a] Average of three determinations.

the ion-sensing membrane and the electrode often leads to unreliable potential readings. Therefore, a number of studies have been focused on the development of ion-to-electron transducer layers to improve the electrode performance. For instance, conducting polymers (CPs) with high electronic and ionic conductivities are widely used as transducer layers.[42] In general, CPs are employed as intermediate layers, and then, the intermediate layers are covered with an ion-selective membrane. In the present work, MWCNT was drop casted on the C-SPE and Cu-microfabricated electrode followed by drying and coating with an ion-selective membrane.[43] The inclusion of MWCNTs provides more stability to the electrical signal and minimizes the potential drift due to the elevated specific surface area that maximizes the electrode double layer capacitance.[44] The solid-contact THZ-ISEs based on MWCNT as an ion-to-electron transducer showed improved potential stability and a low detection limit.

Our initial work was planned to identify the most effective membrane combinations using the conventional inner liquid fabrication procedure. Different plasticizers and ion-exchangers were tried in various combinations to form the sensors I–VI, and preliminary studies showed that O-NPOE was the best plasticizer that provided promising results with TFPB and TCPB. This encouraged us to use it in the subsequent sensors. The sensors VII–X were obtained by introducing two different new ionophores into the sensing matrices to enhance the membrane selectivity. The families of supramolecular calixarene and cyclodextrin are known by their molecular recognition role. A member of each family (CX6 and 2-HP βCD) was added with each ion exchanger to form more selective membranes, as

presented in Table 1. The results in Table 2 show that among the sensors I–X, the sensor VII (O-NPOE/CX6/TFBP) offers an optimum performance regarding linearity, Nernstian slope, and limit of detection. This may be due to TFBP acting as an effective ion exchanger and the presence of a lipophilic additive that allows free movement of the large host–guest molecule CX6–THZ, which increase membrane sensitivity and selectivity.

We also used disposable screen-printed electrodes as an integral part of our study to develop a cost-effective sensing platform for the determination of THZ. A Cu-microfabricated electrode was prepared by combining the photolithography and drop-casting techniques,[45] and a carbon-SPE and C-SPE/MWCNTs were prepared by the drop-casting technique.[37] The sensors XI–XIII were separately used as a solid contact for the optimized PVC-sensing membrane. The optimum combination of the sensing components (sensor VII) was applied on the C-SPEs.

3.2. Potentiometric performance

The potentiometric behavior of the assembled sensors was determined in the concentration range of 1×10^{-2} to 1×10^{-8} mol L^{-1}. The proposed electrodes showed a good Nernstian response over a wide concentration range, reaching 10^{-6} M, whereas the sensors VII and XIII showed better sensitivity, reaching 10^{-7} M, as shown in Table 2. The inclusion of MWCNTs provided more stability to the electrical signal and minimized the potential drift due to the elevated specific surface area that maximized the electrode double layer capacitance. Low detection limit of conventional ISEs is often impaired by transmembrane ion fluxes from the internal filling liquid to the sample.[44] Minimizing this ion flux lowers the detection limits to sub-nanomolar levels. Although all-solid-state ISEs do not contain inner filling solutions as large reservoirs of the primary ions, the sensor XIII was as sensitive as sensor VII (conventional ISE). This can be due to the leakage of primary ions from the sensing material to the sample, which was confirmed by atomic absorption spectrometry.[46] Improving the sensitivity of solid-contact membranes, especially of those containing conducting polymers, can be attained by proper conditioning protocols.[47] Conditioning of the electrodes is preferably accomplished by two steps: a drug ion at higher concentrations, such as 1 mM or 1 μM, is used to substitute the counterions initially existing in the freshly prepared sensing material with analyte ions; subsequently, a second step of conditioning of the sensing material in an analyte solution of significantly lesser concentration, such as 1 μM or 1 nM, is performed.[47] This is an efficient robust approach to attain

Table 5 Determination of THZ in spiked aqueous humor by the suggested sensors

Spiked aqueous humor	Sensors	Found%[a]	R.S.D%
10^{-5} M	Sensor VII	100.77	1.436
	Sensor XI	99.405	1.205
	Sensor XII	100.416	1.168
	Sensor XIII	100.673	0.839
10^{-6} M	Sensor VII	99.63	0.85
	Sensor XI	99.065	1.2009
	Sensor XII	100.046	1.455
	Sensor XIII	99.257	0.828

[a] Average of three determinations.

minimal detection limits for all SC-ISEs employing PVC.[48,49] Therefore, the sensor XIII presented superior performance in terms of slope, sensitivity, durability, and easy handling, whereas the sensor VII presented the best performance in terms of sensitivity and accuracy. All performance parameters, such as linearity ranges, accuracy, and precision, for the proposed sensors are presented in Table 2.

3.3. Effect of pH

For the quantitative applications of these potentiometric sensors, studies were conducted to achieve optimum experimental conditions. The potentiometric response of the sensors (VII and XI–XIII) was studied by making calibrations at the pH values of 3–8 using phosphate buffer and keeping all other variables, including the composition of the sensors, constant (Fig. 4). The results show that the sensors work adequately in the pH range 3–8 as no intrusion from H^+ or OH^- was observed. Moreover, it was observed that at pH values higher than 8, non-Nernestian slopes were obtained due to the presence of the non-ionized form of the drug ($pK_a = 10.5$)[50] besides the degradation product of the drug.[15] The working pH during this study was chosen to be 6 because this value is close to the normal pH of the aqueous humor[51] and the pharmaceutical preparation[52] that represent the target application.

3.4. Response time and lifetime

Active response time is a critical aspect for analytical techniques. The time required for the sensors to reach values within ± 2 mV of the final stable potential was less than 15 seconds at concentrations higher than 10^{-5} mol L^{-1}; however, the time required to reach a constant equilibrium potential in more diluted samples was longer (<20 s), as presented in Table 2. The potential was stable for at least five minutes, and then, slow divergence of potential could be observed. The sensors were tested for 12 weeks, during which the electrodes were frequently used (one hour per day). Loss of plasticizer, ion carrier (ionophore), or ionic site from the polymer sensing film due to their discharge into the sample is the principal reason for the reduced life times of the typical liquid contact membrane sensors.[53] This hampers the long-term stability of solid contact sensors, especially the sensor XIII that contains the hydrophobic MWCNTs (Table 2).

3.5. Selectivity behavior

To prove the selectivity of the developed sensors and that the electrode can distinguish between THZ and other ions, we measured the selectivity of the THZ ISEs against ions that are abundant in many biological fluids as well as against possible co-administered drugs (Table 3). The values of the selectivity coefficient prove that the developed sensors are highly selective for THZ even in the presence of other ions. The satisfactory selectivity of the constructed sensors can be credited to the fine optimization of the cation exchanger–plasticizer complex, and most importantly, optimization of the ionophore. The selectivity of the proposed sensors originates from different combined factors. As is known, for the

detection of a certain ion using an ion-selective electrode, this ion should be transferred to the external part of the electrode.[40] At first, the movement of the THZ ions, specifically toward the electrode, is based on the concentration gradient (THZ concentration in the bulk of the solution is higher than that on the electrode surface). Then, the entry of the target ion into the external layer of the membrane depends on the presence of an ion exchanger that has higher affinity, which is enhanced by a 24 h soaking step as preconditioning, to THZ than to more hydrophilic ions. Subsequently, the most suitable ionophore, determined after optimization, acts as a carrier to help in the entry of THZ to the outer layer of the electrode. After this, the developed potential is transduced through the effective MWCNT layer. All these factors help the electrode in the specific detection of THZ. Based on the high selectivity of the presented sensors, four superior sensors (VII and XI–XIII) were applied to quantify THZ in a pharmaceutical formulation and aqueous humor.

3.6. Sensor potential stability and water layer test

SC-ISEs are proven to have reasonable short-term stability and acceptable potentiometric characteristics; however, their long-standing stability might be impaired by the development of
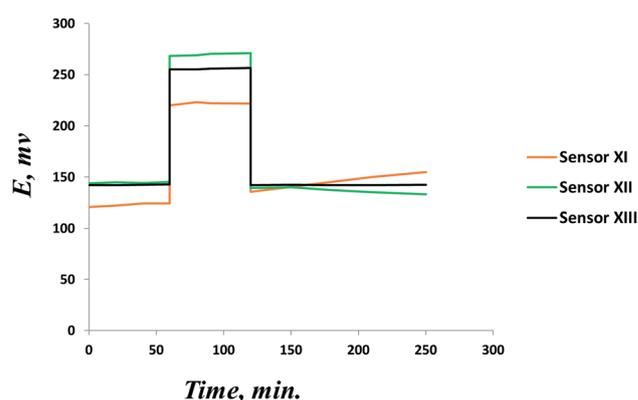

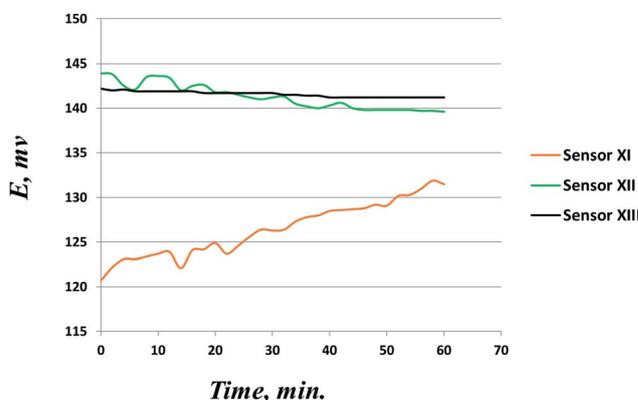Fig. 5 Water layer test for the sensors XI–XIII.

Fig. 6 Potential drift over time for the sensors XI–XIII.

Table 6 Statistical analysis of the results obtained by the proposed sensors and those obtained by the official method[59] for the analysis of THZ in its pure powdered form

Method	Sensor VII	Sensor XI	Sensor XII	Sensor XII	Official method
Mean	99.98	100.20	100.71	99.72	100.030
Variance	0.45	0.37	0.72	0.52	0.335
n	5.00	5.00	5.00	5.00	5
t test	0.731(2.306)	0.454(2.306)	1.482(2.306)	0.482(2.306)	
F test	1.3 (6.39)	1.09(6.39)	2.14(6.39)	0.64(6.39)	

an aqueous layer underneath the polymer film. To test the long-term stability of the proposed sensors, their examination by the formerly created potentiometric aqueous layer test was necessary.[54] This test depends on the sensing potential drifts upon altering the THZ solution (0.001 mmol L^{-1}) to a very concentrated intervening ion solution gatifloxacin (0.1 mmol L^{-1}) and then sensing this THZ solution again. If an aqueous layer is developed underneath the membrane film, the ionic structure of the aqueous layer will also be altered by the through-membrane ion fluxes, generating noticeable potential drifts. Fig. 5 shows the aqueous layer test results for the sensors XI–XIII; for the sensor XIII, no considerable drift in the potential responses was observed, indicating the absence of aqueous layers for the sensor XIII; however, a potential drift was observed for the sensors XI and XII. Furthermore, same results were achieved by examining the constancy of the potential response of the designed sensing platforms in the THZ solution (0.001 mmol L^{-1}) over a 60 minute interval, as shown in Fig. 6. This may be due to the hydrophobic character of the MWCNT nanocomposite that prevents the formation of the water layer, thereby providing long-term stability to the developed sensor. This result is in good accordance with the recently reported results.[55,56] Gatifloxacin was chosen as an interferent because it has a positive center[57] at the studied pH and high lipophilicity and it is a potential drug co-administered with THZ.

Table 7 Penalty points for the greenness assessment of the proposed potentiometric method as compared to those of the official method

| Hazard | Penalty points | |
	Proposed method	Official method
Reagents		
Tetrahydrofuran	6	
Glacial acetic acid		8
Acetic anhydride		12
Perchloric acid		16
Phosphate buffer	0	
Instruments		
Energy (≤0.1 kW h per sample)	0	0
Occupational hazard	0	0
Waste	6	8
Total penalty points	12	44
Analytical Eco-scale total score	88[a]	56[a]

[a] >75 represents excellent green analysis, >50 represents acceptable green analysis, <50 represents inadequate green analysis.

3.7. Potentiometric determination of THZ in pharmaceutical formulations

The proposed sensors VII, XI–XIII were successfully applied for the quantitation of THZ pharmaceutical formulations with no interference of the co-formulated drugs or excipients (Table 4).

3.8. Potentiometric determination of THZ in rabbit aqueous humor

After application in the eye, THZ is delivered in small quantities through the anterior chamber to the aqueous humor; thus, a sensitive and selective method is required to determine THZ in the aqueous humor. Rabbit aqueous humor was spiked with 10^{-5} and 10^{-6} M THZ, and all the presented sensors were found to be reliable and provided stable results with acceptable accuracy and high percentage recovery of THZ without preliminary extraction procedures (Table 5).

3.9. Statistical analysis

No significant difference was found between the proposed potentiometric approach and the official compendial assay method in terms of accuracy and precision (Table 6).

3.10. Greenness assessment of the proposed method: analytical Eco-scale[58]

The calculation of the Eco-scale is based on providing penalty points to any aspect that does not conform to the perfect green technique, where the perfect green practice has an Eco-scale value of 100, an excellent green analysis scores >75 Eco-scale, an acceptable green analysis scores >50, and if the method scores <50, it will be considered as inadequate green analysis.[58] The penalty points for the proposed electrochemical method as compared to those for the official method[59] are shown in Table 7, where the proposed approach shows an excellent Eco-score, whereas the official method, which is non-aqueous titration,[59] has a barely acceptable score due to the use of hazardous organic solvents in large quantities. Moreover, there has been a recent interest to replace THF with a greener solvent that can improve the Eco-score of the potentiometric methods.

4. Conclusion

Herein, a strategy is presented to develop optimized ion-selective membrane sensors for the assessment of THZ. The suggested approach demonstrates a structured optimization for

the assembly of SC-ISEs based on MWCNTs, and a comparable response was obtained using the liquid contact and the solid contact membranes. The sensors were successfully used for the determination of THZ in a pharmaceutical formulation and rabbit aqueous humor. The pharmaceutical components and additives frequently employed in eye drops did not reveal any interference. As revealed by the high precision and accuracy of the recoveries of the spiked aqueous humor, there was no intrusion from the aqueous humor electrolytes. This application could be further investigated in the future to perform bio-analytical method validation of the proposed sensors for the quantitation of THZ in biological fluids. The proposed sensor with MWCNTs offers a reasonable cure to the problem of potential drift, which probably originates from the formation of a water layer at the interface between the polymer membrane and the electrode, affecting the long-term stability of the SC-ISEs. The designed approach is greener than the official method according to the calculations conducted *via* the Eco-scale tool. The constructed sensing platforms have the advantages of rapid response and no requirement of drug pretreatment, preparation steps, and pre-separation. The presented analytical technique has a low cost; therefore, these sensors can be used for the routine analysis of THZ in quality control laboratories.

Conflicts of interest

The authors declare no competing financial interest.

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