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Development of Membrane Electrodes for the Specific Determination of Tetryzoline Hydrochloride in Presence of its Degradation Product in Pharmaceutical Formulations and Biological Fluids

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Abstract- Membrane selective electrodes were used to determine tetryzoline hydrochloride (TZH) in pure form, pharmaceutical preparations and in biological fluids. The membrane selective electrodes include construction of water insoluble ion-association complexes. The TZH ion exchangers were formed using tetraphenyl borate (TZH-TPB), phosphomolybdic acid (TZH-PMA) and phosphotungstic acid (TZH-PTA), in a plasticized PVC (polyvinyl chloride) matrix, using dibutyl phthalate (DBP) or dioctylphthalate (DOP) as a plasticizer. The performance characteristics of the developed sensors were evaluated according to IUPAC recommendations. The developed sensors showed good responses but the best electrochemical characteristics and selectivity coefficients were achieved with TZH-TPB sensor using DBP as a plasticizer, where the linear responses of TZH was found within the concentration ranges of 10^{-6} to 10^{-2} mol/L and Nernstian slope was calculated to be of 56.8 mV/ decade at 25 °C, over the pH range of 5–9. The suggested method was used to determine TZH in synthetic mixtures, pharmaceutical formulations and in presence of its alkali degradation product. The proposed sensors displayed useful analytical characteristics for the determination of TZH in biological fluids such as rabbit aqueous humor and human plasma. The later application can be used to detect oral TZH poisoning in children. The obtained

results were statistically compared with the official method, showing no significant difference with respect to accuracy and precision.

Keywords- Tetryzoline, Aqueous Humor, Human plasma, Degradation

1. INTRODUCTION

Tetryzoline HCl (TZH) 4,5-Dihydro–2-(1,2,3,4–tetrahydro–1–naphthalenyl)-1*H*-imidazole hydrochloride is an imidazoline derivative. It is a sympathomimetic agent with marked alpha adrenergic activity exhibiting a vasoconstrictor effect, so it is used as conjunctive and nasal decongestant. Tetryzoline HCl (TZH) is combined together with antibiotics, corticosteroids and anti-allergics to formulate anti-infective eye preparations to treat acute and sub-acute conjunctivitis, keratitis and corneal ulcers [1]. Several accidental cases were reported about that accidental ingestion happened by children of eye drops and nasal decongestant sprays, containing TZH, which resulted in serious conditions [2-5]. Gas Chromatography-Mass Spectrometry (GC-MS) method was reported for the determination of TZH in urine and blood samples [6] as a screening test for TZH poisoning.

Tetryzoline HCl (TZH) is subjected to alkali degradation to produce N-(2-ethylamino)-1,2,3,4-tetrahydro -1-naphthamide [7-10]. The chemical structure for TZH and its pathway of degradation are shown in Fig. 1.

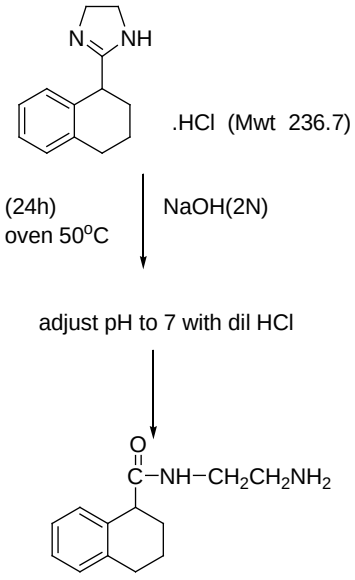

Fig. 1. The chemical structure for TZH and its pathway of alkali-induced degradation

Different analytical techniques were reported for TZH determination in pharmaceutical preparations and in biological fluids such as: colorimetric determination [11], spectrophotometry [12-16], HPTLC [17], HPLC [7-10,18-19] and gas chromatography [6].

Revealing the literature review, no potentiometric method was reported for determination of TZH.

So the aim of this work was to develop a simple, accurate, precise and sensitive potentiometric method for determination of TZH using ion selective membrane electrodes in bulk, pharmaceutical formulations and in biological fluids by ion selective electrodes. The potentiometric method described the fabrication of the ion selective membrane electrodes, through the use of tetraphenylborate (TPB), phosphomolybdic acid (PMA) and phosphotungestic acid (PTA) as anionic exchangers for the development and optimization of novel sensors for the determination of TZH.

The optimized sensors were used for the determination of TZH in bulk powder, different pharmaceutical formulations and in biological fluids such as rabbit aqueous humor and human plasma, where the optimized sensor can be used to detect topical TZH present in aqueous humor and oral TZH poisoning in children. TZH was also determined in the presence of its alkali-induced degradation product and so this method could be used as a stability-indicating method for TZH in different pharmaceutical formulations.

2. EXPERIMENTAL

2.1. Apparatus

A Jenway digital ion analyzer model 3505 (Jenway, UK) with Ag/AgCl double junction reference electrode (Aldrich, USA) was used for potential measurements. A Jenway pH glass electrode (Jenway, UK) was used for pH adjustments.

2.2. Chemicals and reagents

Pure sample. Tetryzoline hydrochloride (TZH) was supplied by Sigma–Aldrich (USA). Its purity was found to be 100.06±0.59 by the official method [20]. Chloramphenicol and prednisolone acetate were kindly supplied by Sigma Pharmaceutical Industries Limited, Al-Monofeya, Egypt. Ofloxacin, dexamethasone sodium phosphate and 80% solution of benzalkonium chloride were kindly supplied by Egyptian International Pharmaceutical Industries Co. (EIPICO), Cairo, Egypt.

Market samples. Loxtra® eye drops, labeled to contain 3 mg of ofloxacin, 2 mg of prednisolone acetate, 0.4 mg of TZH and 0.05 mg of benzalkonium chloride per 1 mL; Croma® eye drops, labeled to contain 40 mg of sodium cromoglicate, 0.5 mg of TZH and 0.1 mg of benzalkonium chloride per 1 mL. Both eye drops were manufactured by Jamjoompharma, Kingdom of Saudi Arabia. Orchadexoline® eye drops, labeled to contain 5 mg of chloramphenicol, 1 mg of dexamethasone sodium phosphate, 0.25 mg of TZH and 0.02 mg benzalkonium chloride per 1 mL, was manufactured by Orchidia Pharmaceutical Industries, Al-Obour city, Egypt. The samples were purchased from the local market.

Chemicals and Reagents. All chemicals and reagents used were of analytical reagent grade. High molecular weight polyvinyl chloride (PVC), tetraphenylborate 99.99% (TPB), phosphomolybdic acid 99.99% (PMA) and phosphotungestic acid 99.97% (PTA), dioctyl phthalate (DOP), dibutyl phthalate (DBP) and tetrahydrofuran (THF) were obtained from Aldrich, USA. Hydrochloric acid, sodium hydroxide scales, potassium chloride, calcium chloride, hydroxypropyl methyl cellulose, macrogol and urea were obtained from El-Nasr pharmaceutical chemicals, Cairo, Egypt. Phosphate buffer solution (prepared according to B.P. using 0.2 M potassium dihydrogen phosphate). Fresh aqueous humor was extracted from albino rabbits, while plasma sample (collected from healthy volunteer) were obtained from VACSERA, Egypt.

2.3. Standard solutions

Stock solution. TZH was prepared in distilled water (10^{-2} M).
Working solutions. They were freshly prepared by dilution from the stock solution with distilled water (10^{-7}-10^{-3} M).

2.4. Procedures

2.4.1. Precipitation of the ion exchangers

In three different beakers, ten ml aliquot of 10^{-2} M aqueous standard TZH solution was treated separately with 10 ml of aqueous 10^{-2} M of each of TBP, PMA and PTA solutions, respectively. The prepared solutions were shaken well for 5minutes.The precipitates formed were filtered using Whitman filter papers, washed with cold water till chloride free (tested by $AgNO_3$ solution), dried at room temperature (≈25°C) and then ground to fine powder. The formation and purity of the ion-associates and the chemical compositions of the precipitates were checked by elemental analysis for carbon, hydrogen and nitrogen. The results are given in Table 1.

Table 1. The elemental analysis of ion-associates

Ion-associates	Tentative formulae	Percentage %	C%	N%	H%
TZ - TPB	$[C_{13}H_{16}N_2].[C_{24}H_{20}B]$	**Found**	85.62	5.47	7.34
		Calculated	85.38	5.38	7.16
TZ - PM	$[C_{13}H_{16}N_2]_3.[PMo_{12}O_{40}]$	**Found**	19.41	3.56	2.18
		Calculated	19.31	3.46	2.12
TZ - PT	$[C_{13}H_{16}N_2]_3.[PW_{12}O_{40}]$	**Found**	13.58	2.35	1.59
		Calculated	13.46	2.41	1.48

2.4.2. Fabrication of PVC based membrane sensors

For the preparation of ISE sensor, in separate glass petri dishes (5 cm diameter) 10 mg of the ion exchangers (TZ-TBP), (TZ-PM) and (TZ-PT) were separately mixed with 0.35 ml of DBP or DOP. Amounts of 0.19 g PVC were added to each petri dish and then the mixtures were dissolved in 5 ml THF. The petri dishes were covered by filter papers and left to stand overnight to allow solvent evaporation at room temperature. These ratios of components added will form a master membrane with a thickness of 0.1 mm. The fabrications of the six ISE electrodes are described in Table 2. From the formed master membranes, disks (≈10 mm diameter) were cut using a cork borer and pasted using THF to interchangeable PVC tips that were clipped into the end of the electrodes glass bodies. Equal volumes of 10^{-2} M TZH and 10^{-2} M KCl were mixed and this obtained solution was used as an internal reference solution. Ag/AgCl wire (1mm diameter) was immersed in the internal reference solution as an internal reference electrode. The electrodes were preconditioned by immersing in 10^{-2} M TZH solution for 24 h. The electrochemical cell for potential measurements was: Ag/AgCl (internal reference electrode)/ 1.0×10^{-2} M TZH solution, 1.0×10^{-2} M KCl (internal reference solution)//PVC membrane//test solution (pH 6-8) & (pH 4-6)//Ag/AgCl double junction reference electrode. The electrodes were stored in distilled deionized water between measurements.

Table 2. The composition of the six fabricated sensors

Sensor	Ion-associate	Plasticizer
1	TZH - TPB	DBP
2	TZH - TPB	DOP
3	TZH - PM	DBP
4	TZH - PM	DOP
5	TZH - PT	DBP
6	TZH - PT	DOP

2.4.3. Sensors calibration

The conditioned sensors were calibrated by separately transferring 50 ml aliquots TZH solutions prepared in distilled water with concentration range of (1×10^{-7}–1×10^{-2} M) into a series of 100 ml beakers starting from the low to the high concentrations. The membrane sensors in conjunction with a reference electrode were immersed in each solution, allowed equilibrating with constant stirring using a magnetic stirrer, then recording the stable potential within ±2 mV. The electrode potential (EMF) was plotted versus each negative

logarithmic concentration of TZH. The response time of the investigated electrodes was calculated.

2.4.4. Effect of pH

The effect of pH on the potential values of the six investigated sensors was studied over pH range of 3-10 at one pH interval by using 10^{-4} M and 10^{-3} M TZH solutions. The pH was gradually increased or decreased by adding aliquots of dilute sodium hydroxide or dilute hydrochloric acid solutions respectively. The potential obtained at each pH value was recorded.

2.4.5. Sensors selectivity

The potentiometric selectivity coefficient –log $K^{pot.}$ (Primary ion, interferent) was used to evaluate the extent to which a foreign ion would interfere with the response of an electrode to its primary ion. Selectivity coefficients were calculated by the separate solutions method, where potentials were measured for 10^{-3} M aqueous TZH solution and then for 10^{-3} M aqueous interferent solution separately then potentiometric selectivity coefficients were calculated using the following equation:

$- \log K^{pot.}(\text{Primary ion, interferent}) = (E_{TZH} - E_M) / \text{Slope}$

Where E_{TZH} is the potential measured in 10^{-3} M TZH solution and E_M is the potential measured in 10^{-3} M interferent solution.

2.4.6. Application to pharmaceutical dosage forms

2.4.6.1. Loxtra® eye drops

Four milliliters of the eye drops were transferred into 25 mL volumetric flask, the volume was completed with distilled water to get 64 μg mL^{-1} of TZH, and then further dilution was made to obtain a solution of 320 μg mL^{-1} of TZH.

2.4.6.2. Croma® eye drops

Three milliliters of the eye drops were transferred into 25 mL volumetric flask, the volume was completed with distilled water to get 60 μg mL^{-1} of TZH, and then further dilution was made to obtain a solution of 300 μg mL^{-1} of TZH.

2.4.6.3. Orchadexoline® eye drops

Five milliliter of the eye drops were transferred into 10 mL volumetric flask, the volume was completed with distilled water to obtain a solution of 125 μg mL^{-1} of TZH.

The procedure was then completed as described under section *2.4.3*. Six replicates of each experiment were done. From the recorded potential, the concentration of TZH was calculated

from the corresponding regression equation. The standard addition technique was applied by adding different known concentrations of pure standard TZH to the pharmaceutical formulation before proceeding in the previously mentioned procedure.

2.4.7. Preparation and determination of the alkali-induced degradation product of TZH

A sample of 50 mg of TZH was weighed into 100 mL volumetric flask and dissolved in 20 mL of distilled water. Five milliletrs of 10 M NaOH were added and the solution was placed in an oven at 50 ^{o}C for 24 h. the pH was then adjusted to 7 with 10 M HCl and the volume was completed to the mark with distilled water [8]. Further dilution was done with distilled water to form concentrations of (10^{-4} M and 10^{-3} M). The structure of the induced degradation product was elucidated using the IR spectrometry.

2.4.8. Analysis of laboratory-prepared mixtures

Aliquots of standard drug solution (10^{-4} M and 10^{-3} M) were mixed with its degradation product (10^{-4} M and 10^{-3} M), respectively, to prepare solutions of TZH containing 10%, 30%, 50%, 70% and 90% of degradation product. The EMF values of these laboratory-prepared mixtures were recorded and results were compared with the calibration plot.

2.4.9. Determination of TZH in spiked rabbit aqueous humor

Five albino rabbits were used to obtain a fresh aqueous humor. Two drops of 0.4% solution of benoxinate HCl (Local anesthetic) were instilled into rabbit's eye. Samples of aqueous humor were immediately removed from the anterior chamber of each eye using a 26-gauge needle attached to 1 ml tuberculin syringe. The procedure was repeated 2 times a day for about 3-5 days till the wanted volume was collected. The samples were stored frozen until the experiment was carried out [21]. After removal of aqueous humor samples at each time interval, the ocular surface was irrigated with isotonic phosphate buffered saline and dried with soft tissue. Half milliliter of the working solutions of 10^{-3} M and 10^{-4} M standard TZH solution were separately introduced to two 5-ml volumetric flasks and the volume was completed with the collected aqueous humor, and then transferred into test tube and vortex for 30 seconds. The membrane sensors were immersed in these solutions and washed with water between measurements. The produced EMF was measured by the proposed sensors and the concentration of TZH was calculated from the corresponding regression equation.

2.4.10. Determination of TZH in spiked human plasma

Half milliliter of the working solutions of 10^{-3} M and 10^{-4} M standard TZH solution were separately introduced to two 5-ml volumetric flasks and the volume was completed with plasma, and then transferred into test tube and vortex for 30 seconds. The membrane sensors

were immersed in the obtained solution and washed with water between measurements. The produced EMF was measured by the proposed sensors and the concentration of TZH was calculated from the corresponding regression equation.

3. RESULTS AND DISCUSSION

The rapid growth in analytical chemistry techniques is necessary to match the development of a wide variety of science and technology approaches. In the last three decades, Potentiometric sensors have been used for specific determination of many drugs [22-24]. Ion selective electrodes (ISEs) possess much advantage over traditional methods of analysis and provide accurate, reproducible, fast and regular selective determination of various ionic species [23,25-26]. In addition, ISEs allow non-destructive, on line monitoring of particular ions in a small volume of sample without pretreatment [21,24,27].

The present work evaluated the possibility of quantitative determination of TZH by ISE sensors with ion exchanger TPB, PMA and PTA in its composition using PVC. It has been reported that PVC matrix is a regular support and acts as a polymeric matrix to immobilize the sensors and to attain the formation of highly stable complexes. Nevertheless, its use created a need for plasticization and places a constraint on the choice of mediator [28]. In the present study, the use of the plasticizers, dibutyl phthalate (DBP) and dioctyl phthalate (DOP), were studied for the fabrication of the proposed membrane sensors. Both plasticizers adjusted the permittivity of the final organic membranes and mobility of the ion exchanger sites. The membranes constituents were dissolved in THF that was slowly evaporated at room temperature leading to membrane formation.

The proposed sensors were used for the determination of TZH in bulk powder, different pharmaceutical formulations and in biological fluids such as rabbit aqueous humor and human plasma. The proposed membrane sensors could be utilized in a stability indicating method for the determination of TZH in presence of its alkali-induced degradation product.

3.1. Performance characteristics of TZH sensors

It was found that the three ionic exchangers have low solubility product and suitable grain size. TZH was found to react as a monovalent cation, as it formed 1:1 ion association complex with TPB, while it formed 3:1 ion association complex with PMA and PTA. This was proven by elemental analysis, as shown in Table 1, and by the obtained Nernstian slopes. The electrochemical performance characteristics of the investigated TZH sensors were evaluated according to the IUPAC recommendation data [29] and the results were shown in Table 3. Typical calibration plots are shown in Fig 2. The slopes of the calibration plots showed deviation from the ideal Nernstian slope (60 mV), which is due to the electrodes responding to the activity of the drug cation rather than its concentration. Moreover, sensors 1

and 2 (TZH-TPB) showed the closest value to the ideal Nernstian slope, 56.8 and 52.9 mV/decade, respectively. The sensors displayed constant potential readings for day to day measurements, and the calibration slopes did not change by more than ±2 mV/decade over the stability periods of the developed sensors, where sensors 1 and 2 (TZH-TPB) showed the longer stability period (40 days). The slopes of the calibration plot did not change significantly but show a gradual decrease in sensitivity.

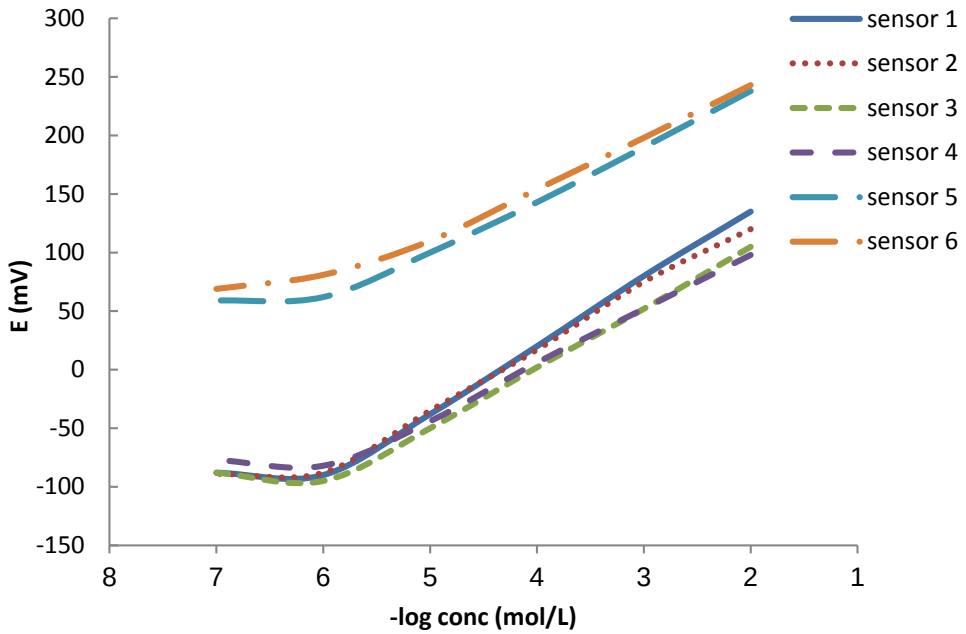

Fig. 2. Profile of the potential in mV versus −log concentration of TZH in mol/L obtained with the proposed sensors

Sensors from 1 to 4 (TZH-TPB and TZH-PM) showed good sensitivity, where linearity was obtained in the range of (10^{-6}-10^{-2} M); while sensors 5 and 6 (TZH-PT) fell short in the limit of linearity (10^{-2}-10^{-5} M). The limits of detection (LOD) of the six sensors were estimated, where sensor 1 (TZH-TPB using DBP as plasticizer) showed the lowest LOD, as it could detect TZH in very dilute solutions down to 8.6×10^{-7} mol/L.

Dynamic response time is an important factor for analytical applications of ion-selective sensors. In this study, practical response time was recorded by increasing TZH concentration by up to 10-fold. The required time for the sensors to reach values within ±2 mV of the final equilibrium potential was 10-15 sec for sensors 1 and 2 (TZH-TPB), while for the other sensors; equilibrium was reached in 5-10 sec. The response time increased with increasing the concentrations.

For quantitative measurements with ion selective electrodes, studies were carried out to reach the optimum experimental conditions. Sensors 1 and 2 (TZH-TPB) showed the widest working pH range, where the potential pH profile obtained indicated that the responses of

those sensors were fairly constant over the pH range 5–9. While the others showed narrower working pH range (6-8). The effect of pH on the developed sensors is shown in Fig. 3.

Table 3. Electrochemical response characteristics of the six investigated membrane sensors

Parameters	**Sensor 1**	**Sensor 2**	**Sensor 3**	**Sensor 4**	**Sensor 5**	**Sensor 6**
Slope (mV/decade) [a]	56.8	52.9	50.2	45.3	46.1	44.3
Intercept (mV) [a]	248.6	229.6	203.6	187.2	329.1	331.3
LOD (mol/L) [b]	8.6×10^{-7}	9.0×10^{-7}	9.4×10^{-7}	9.6×10^{-7}	2.3×10^{-6}	3.5×10^{-6}
Response time (sec)	10-15	10-15	5-10	5-10	5-10	5-10
Working pH range	5-9	5-9	6-9	6-9	6-8	6-8
Linearity range (mol/L)	$10^{-6} – 10^{-2}$	$10^{-6} – 10^{-2}$	$10^{-6} – 10^{-2}$	$10^{-6} – 10^{-2}$	$10^{-5} – 10^{-2}$	$10^{-5} – 10^{-2}$
Stability (days)	30	30	15	15	10	7
Average recovery [a c]	99.97±0.828	99.86±1.245	99.89±1.180	99.89±1.339	99.92±0.992	99.98±0.768
Correlation coefficient	0.9996	0.9990	0.9993	0.9989	0.9994	0.9995
Ruggedness [a c d]	99.97±0.828	100.12±1.986	99.89±1.180	99.89±1.339	99.92±0.992	99.98±0.768

[a] Average of five determination
[b] Limit of detection (measured by interception of the extrapolated arms of Fig.2)
[c] Recovery percentage ± standard deviation
[d] Jenway digital ion analyzer model 3510 (Jenway, UK) with Ag/AgCl double junction reference electrode (Aldrich, USA) was used for potential measurements.

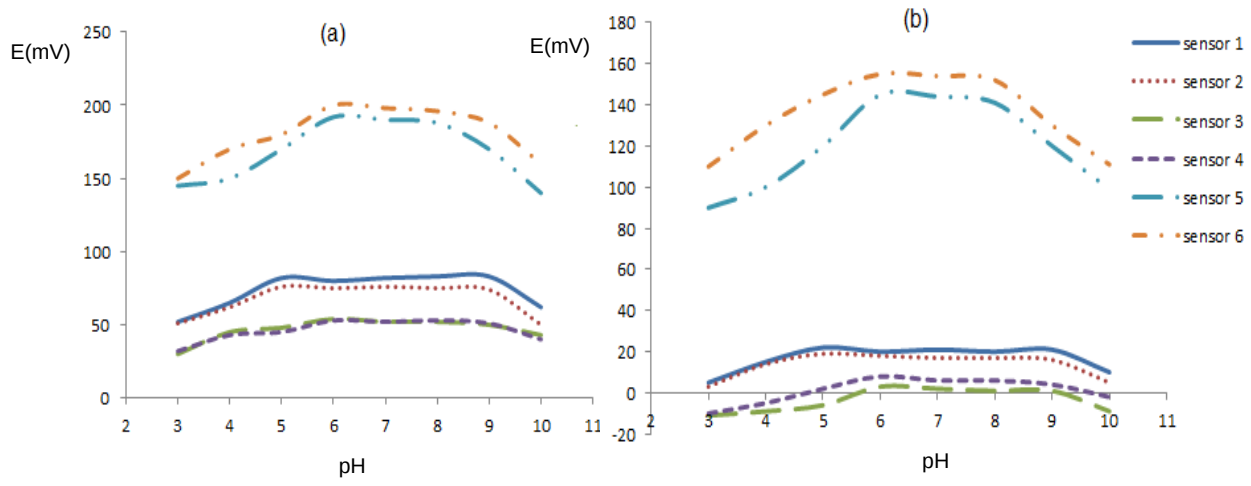

Fig. 3. The effect of pH on the developed sensors using (a) 10^{-3} M TZH solution and (b) 10^{-4} M TZH solution

Table 4 showed the potentiometric selectivity coefficients of the proposed sensors in the presence of the degradation product, co-formulated drugs and excipients in the different

pharmaceutical dosage forms, and some other inorganic cations (K^+, Na^+ and Ca^{2+}) that are usually found in biological fluids. The results reveal that the proposed membrane sensors 1 and 2 (TZH-TPB) display 10 times higher selectivity than sensors 5 and 6 (TZH-PT) for the determination of TZH in presence of its degradation product. While for the other interferents, the proposed sensors showed good selectivity, but sensors 1 and 2 (TZH-TPB) showed the highest selectivity of all.

As shown from the previous results, the best electrochemical characteristics and selectivity coefficients were achieved with TZH-TPB ion exchanger using DBP and DOP as a plasticizers, which are sensors (1 and 2) respectively. So both sensors (1 and 2) were applied for the determination of TZH in presence of its induced degradation product. Also they were applied for the determination of TZH in the different pharmaceutical formulations and biological fluids.

Table 4. Potentiometric selectivity coefficients ($K^{pot.}$) of the six proposed sensors using the separate solutions method

Interferent	Sensor 1	Sensor 2	Sensor 3	Sensor 4	Sensor 5	Sensor 6
TZH degradation product	0.99×10^{-1}	1.31×10^{-1}	5.35×10^{-1}	6.21×10^{-1}	1.12	1.09
Hydroxypropyl methyl cellulose	1.82×10^{-2}	1.76×10^{-2}	2.1×10^{-2}	6.5×10^{-2}	9.1×10^{-2}	1.2×10^{-1}
Macrogol	3.1×10^{-2}	2.7×10^{-2}	4.1×10^{-2}	5.4×10^{-2}	1.8×10^{-1}	1.55×10^{-1}
NaCl	3.11×10^{-3}	4.2×10^{-3}	3.5×10^{-3}	2.8×10^{-3}	1.76×10^{-2}	2.11×10^{-2}
KCl	5.4×10^{-3}	6.5×10^{-3}	7.6×10^{-3}	5.5×10^{-3}	9.8×10^{-3}	1.2×10^{-2}
$CaCl_2$	4.7×10^{-3}	5.6×10^{-3}	4.2×10^{-3}	6.4×10^{-3}	8.8×10^{-3}	1.01×10^{-2}
Urea	2.1×10^{-2}	3.08×10^{-2}	3.1×10^{-2}	5.8×10^{-2}	8.7×10^{-2}	9.9×10^{-2}
Benazlkonium chloride	1.92×10^{-1}	2.05×10^{-1}	2.93×10^{-1}	2.96×10^{-1}	4.12×10^{-1}	1.43×10^{-1}
Ofloxacin	5.3×10^{-3}	6.6×10^{-3}	7.1×10^{-3}	6.9×10^{-3}	8.1×10^{-3}	1.23×10^{-2}
Prednisolone acetate	6.1×10^{-3}	7.2×10^{-3}	6.7×10^{-3}	8.8×10^{-3}	2.13×10^{-2}	6.43×10^{-2}
Chloramphenicol	4.7×10^{-3}	5.2×10^{-3}	5.6×10^{-3}	9.2×10^{-3}	7.5×10^{-3}	2.14×10^{-2}
Dexamethasone sodium phosphate	7.1×10^{-3}	6.8×10^{-3}	5.5×10^{-3}	7.1×10^{-3}	3.31×10^{-2}	7.45×10^{-2}

3.2. Potentiometric determination of TZH in pharmaceutical formulations

TZH is present in very low concentrations in the selected pharmaceutical formulations, which requires a sensitive and selective method for its determination. The proposed sensors (1 and 2) were applied for the analysis of TZH pharmaceutical formulations in aqueous solutions, and the results proved the applicability of the proposed sensors for the determination of pharmaceutical formulations containing TZH with no interference of the co-formulated drugs or excipients. These data were shown in Table 5.

Table 5. Determination of TZH in different pharmaceutical formulations by the sensors 1 and 2

Pharmaceutical formulations	**Sensor 1**	**Sensor 2**
	Recovery (%) ±S.D. *	
Loxtra® eye drops	100.74±0.904	99.70±1.103
Croma® eye drops	97.99±0.782	98.00±0.731
Orchadexoline® eye drops	99.36±0.909	99.68±1.653

* The recovery percentages are the average of five determinations.

3.3. Potentiometric determination of TZH in the presence of its alkali-induced degradation product

The alkali-induced degradation was tested by TLC till complete degradation product was obtained. The structure of the degradation product was elucidated by IR spectroscopy as shown in Fig. 4, where stretching bands, characteristic to the reported structure of the degradation product [7] , appeared at 3459 cm^{-1} (N−H group) and at 1637 cm^{-1} (C=O group). Table 6 shows the results obtained upon analysis of synthetic mixtures containing different ratios of intact drug and degradation product varying from 100:0 to 10:90. The result shows that sensors (1 and 2) can be successfully used for selective determination of intact drug in the presence of up to 90% of its degradation product. Sensors (5 and 6) were found to be non-selective to TZH in presence of its degradation product, as $K^{pot.}$ was calculated to be>1. So the proposed sensors (1 and 2) can be used as stability-indicating method of TZH in bulk and pharmaceutical formulations.

3.4. Potentiometric determination of TZH in rabbit aqueous humor

As mentioned before, TZH is present in low concentrations in different pharmaceutical formulations, and it will be delivered in small quantities through anterior chamber into the aqueous humor, so a sensitive selective method was required to determine TZH in aqueous humor. Rabbit aqueous humor was spiked with low concentrations of TZH (10^{-5} and 10^{-4} M), and it was found that the sensors (1 and 2) were reliable and gave stable results with very good accuracy and high percentage recovery for the determination of TZH without preliminary extraction procedures, as shown in Table 7. The response times of the proposed

sensors were instant (within 15 sec), so the sensors are rapidly transferred back and forth between the biological samples and the distilled water between measurements to protect the sensing component from adhering to the surface of some matrix components. It is concluded that the proposed sensors can be successfully applied to in vitro studies and for clinical use.

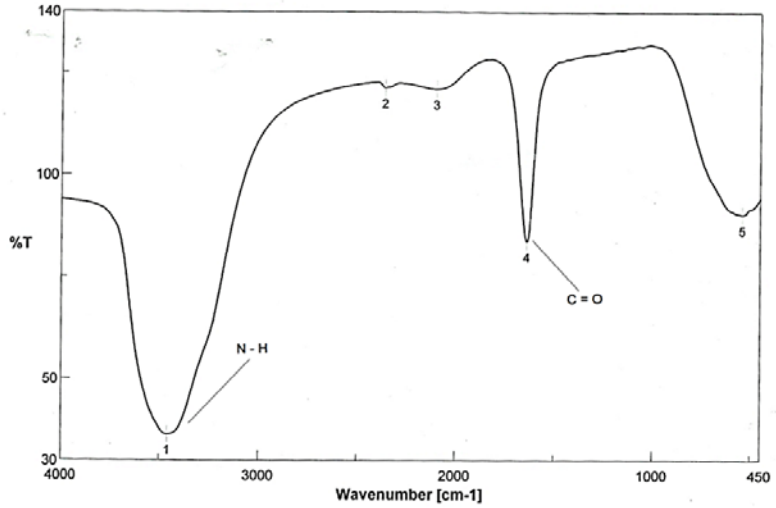

Fig. 4. IR spectrum characteristic to the structure of the alkali-induced degradation product

Table 6. Determination of TZH in laboratory prepared mixtures containing different ratios of TZH and its alkali-induced degradation product by the proposed sensors 1 and 2

% Drug : degradation product	**Sensor 1**	**Sensor 2**
	Recovery (%) ± S.D. *	
100:0	100.13±0.563	99.56±1.621
90:10	99.29±1.210	99.73±0.812
80:20	100.55±0.881	100.11±0.629
70:30	101.34±1.102	99.84±1.210
60:40	99.84±0.982	98.43±0.691
50:50	99.92±0.855	100.30±0.779
40:60	98.33±0.992	101.44±0.684
30:70	99.93±1.193	98.44±1.732
20:80	97.87±1.493	101.46±1.825
10:90	97.49±1.561	97.44 ±1.693

* The recovery percentages are the average of three determinations of each of 10^{-3} M and 10^{-4} M solutions of TZH and its degradation product.

Table 7. Determination of TZH in spiked rabbit aqueous humor and human plasma by the proposed sensors 1 and 2

TZH concentration	Spiked rabbit aqueous humor		Spiked human plasma	
	Sensor 1	Sensor 2	Sensor 1	Sensor 2
	Recovery (%) ± S.D. *			
10^{-4} M	97.92±0.832	98.04±0.754	100.12±0.912	99.32±0.983
10^{-5} M	97.12±1.198	97.16±1.021	98.01±1611	97.77±1.722

3.5. Potentiometric determination of TZH in spiked human plasma

In 2012, the U.S. Food and Drug Administration (FDA) warned the public that accidental ingestion happened by children (5 years of age and younger) of only a small amount (1-2 ml) of over-the-counter (OTC) eye drops and nasal decongestant sprays, containing TZH, could result in serious harm. Through the reported accidental cases, no deaths were reported; however, serious events requiring hospitalization such as coma, decreased heart rate, decreased breathing, and sedation have occurred [4,5]. So, the ion-selective membrane sensor was proposed as a sensitive, selective and low-cost alternate for the reported GC-MS method for the determination of TZH in plasma samples [6].

Table 8. Statistical comparison between the results obtained by the proposed sensors and the official BP methods for the determination of TZH in pure form

Items	Sensor 1	Sensor 2	Sensor 3	Sensor 4	Sensor 5	Sensor 6	Official method [d]
Mean	99.97	99.86	99.89	99.89	99.92	99.98	100.06
Variance	0.686	1.550	1.392	1.794	0.985	0.072	0.353
Standard error of mean (SEM)	0.372	0.557	0.528	0.599	0.496	0.134	0.266
n	5	5	5	5	4	4	5
Student's *t*-test	0.206 [a]	0.319 [a]	0.290 [a]	0.260 [a]	0.255 [b]	0.241 [c]	
***F* value**	1.947 [a]	4.400 [a]	3.952 [a]	5.090 [a]	2.795 [b]	4.910 [c]	

[a] the corresponding tabulated values of *t*=2.306 and F values=6.389 at (P 0.05).

[b] the corresponding tabulated values of *t*=2.364 and F values=6.591 at (P 0.05).

[c] the corresponding tabulated values of *t*=2.364 and F values=9.117 at (P 0.05).

[d] BP method for TZH it is potentiometric titration method.

The results obtained in Table 7, showed that the proposed sensors (1 and 2) can determine TZH in spiked human plasma samples present in very low concentrations (10^{-5} and 10^{-4} M) with high precision and accuracy. So, the ion-selective membrane sensor can be used as a rapid screening tool for the accidental ingestion of TZH, especially in children.

3.5. Statistical analysis

Statistical comparison of the results obtained by the six proposed sensors and the official method for the analysis of TZH was shown in Table 8. The calculated t and F values were less than the theoretical ones indicating that there was no significant difference between the proposed methods and the official methods with respect to accuracy and precision.

4. CONCLUSION

This work presented a study to develop optimized ion-selective membrane sensors for the determination of tetryzoline hydrochloride (TZH). The sensors were successfully used for determination of TZH in the presence of up to 90% of its alkali-induced degradation product, and this proved the specificity of the method. Pharmaceutical components and additives commonly used in eye drops did not show any interference. Thus, the analysis was carried out without prior treatment or extraction. The method was successfully used for the determination of TZH in several pharmaceutical formulations. On application to the biological fluids, it has been found that the sensor 1 and 2 gave stable results, as revealed by high precision and accuracy of recoveries of the spiked aqueous humor or plasma samples indicating no interference from aqueous humor or plasma electrolytes, so the method can be used as screening test for TZH accidental poisoning. The proposed sensors offered advantages of fast response and elimination of drug pretreatment or separation steps in a low-cost analytical technique; therefore they could be used for routine analysis of TZH in quality control laboratories.

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