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Application of Membrane Selective Electrodes for the Determination of Azelastine Hydrochloride in the Presence of its Alkaline Degradant in Eye Drops and Plasma

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Abstract- Azelastine Hydrochloride (AZT) was described by using the ion association complex between these drugs with either sodium tetraphenyl-borate (TPB) or ammonium reineckate (RNC) counter ions. The performance characteristics of the sensors were evaluated according to IUPAC recommendations, reveal a fast, stable and linear response over the concentration range 10^{-5}- 10^{-2} M for AZT. The sensors are used for determination of AZT in eye drops and plasma. The developed method was found to be simple, accurate and precise when compared with the Manufacturer method.

Keywords- Azelastine Hydrochloride, Ion Selective Electrode, PVC Membranes Ammonium Reineckate, Sodium Tetraphenyl-Borate

1. INTRODUCTION

Azelastine-HCl (AZT) is 4-(4-chlorobenzyl)-2-[(4RS)-1-methylhexahydro-1H-azepin-4-yl] phthalazin-1(2H)-one hydrochloride [1].

This medicament is used topically in the symptomatic relief of allergic conditions, including rhinitis and conjunctivitis [2].

The methods available for analysis of azelastine-HCl in pharmaceutical dosage forms and biological fluids include UV-spectrophotometric methods [3], colorimetric methods [4], potentiometric method [5], capillary electrophoresis method [6], high performance thin layer chromatography (HP-TLC) method [7] and high performance liquid chromatography (HPLC) methods [8-15].

The development and application of ion-selective electrodes continue to be of interest for pharmaceutical analysis because these sensors offer the advantages of simple design and operation, reasonable selectivity, fast response, low cost, wide pH working range, reasonable selectivity, broad concentration range, and applicability to turbid and colored solutions.

In the present work, three ion-selective membrane sensors were developed for azelastine hydrochloride (AZT), based on the use of the ion association complexes of these drugs with sodium tetraphenyl borate and ammonium reineckate. The high lipophilicity and remarkable stability of these complexes suggested their selective use as electroactive materials in PVC matrix membrane sensors for the determination of AZT in the presence of its alkaline degradate, excipients, and plasma without the need of preliminary extraction and separation steps. Moreover, they offer a highly sensitive, selective, and convenient technique for the determination of AZT in either pure forms or in pharmaceutical preparations.

2. EXPERIMENTAL

2.1. Instruments

1- Potentiometric measurements were made with a Hanna (Model 211) pH /mV meter. A single junction calomel reference electrode (Model HI 5412) was used in conjunction with the drug sensor.
2- A WPA pH combined glass electrode Model CD 740 was used for pH measurements.
3- Bandelin sonorex, RK 510 S, magnetic stirrer.
4- Silver wire (3 mm diameter) immersed in the internal solutions.

2.2. Reagents and solvents

All chemicals were of analytical grade and bidistilled water was used. Tetrahydrofuran (THF) 99% (Lab scan), high molecular weight (10000) polyvinylchloride (PVC) powder (Aldrich), sodium tetraphenyl borate (NaTPB) (Aldrich), ammonium reineckate (R) and dibutylsebathete (DBS) were obtained from Sigma, phosphate buffer pH 4 was prepared [1] and plasma was supplied by VACSERA (Giza, Egypt). (2-Hydroxy propyl) β-cyclodextrin from Fluka (Steinhein,Germany). Sodium hydroxide, HCl and potassium chloride were purchased from Prolabo (Pennsylvania, USA).

2.3. Samples

2.3.1. Pure samples

Azelastine-HCl (AZT) was kindly supplied from European Egyptian Pharm Co., Egypt, with 99.26±1.11% purity according to manufacturer's method [16].

2.3.2. Market sample

Azelast eye drops ("BN 16209", El-Kahira Pharm and Chem Ind Co., for EPCI, Cairo Egypt) labeled to contain 0.5 mg AZT per 1 mL.

2.3.3. Degraded sample

It was prepared by dissolving 50 mg of AZT in 50 ml 2 M aqueous NaOH and refluxing for 7 h. The solution was neutralized using 2 M HCl, evaporated to dryness on hot plate and the residue was dissolved in 20 ml methanol. The obtained solution was filtered; the methanol then evaporated to get the azelastine degradant in pure powdered form.

Complete degradation was confirmed by spotting on TLC plates using acetonitrile: water: glacial acetic acid (8: 2: 1, by volume) as a developing system. The spots were dried and visualized under UV light at 254 nm. The structure of the degradation product was elucidated by IR spectroscopy.

2.4. Prepared Solutions

2.4.1. Stock (AZT) Standard Solutions

AZT stock solution (10^{-2} M) in either water or phosphate buffer pH 4 were prepared by transferring 0.418 gm of AZT into two separate 100 ml measuring flasks. 50 ml of either water or phosphate buffer pH 4 were added, shaken for few minutes and completed to volume with the same solvent.

2.4.2. Stock (AZT-alkaline degradant) Standard Solutions

It was prepared from complete degradation of 10 mL of 10^{-1} M standard solution of AZT in 2 M NaOH, refluxed for 7 h the degraded sample was neutralized by 2 M HCl to pH 7 and then transferred quantitatively into 100 mL measuring flask and completed to volume with the phosphate buffer.

2.4.3. Working Standard Solutions

AZT working solutions (10^{-5}-10^{-3} M) were prepared by suitable dilution from its stock solution using either water or phosphate buffer pH 4.

2.4.4. Laboratory-Prepared Mixtures

Aliquot portions 1, 2, 3, 5, 6, 7 and 9 ml of AZT from its stock solution 10^{-2} M was transferred accurately to a series of 10 ml measuring flasks. Aliquot portions from its alkaline

degradant 10^{-2} M solution were added to prepare mixtures containing 10%, 30%, 40%, 50%, 70%, 80% and 90% degradation, respectively.

2.5. Procedures

2.5.1. Precipitation-Based Technique for the Preparation of PVC-Membrane Sensor (Sensor 1 and 2)

10 ml of 10^{-2} M AZT aqueous solution was mixed with either 10 ml of a saturated aqueous solution of sodium tetraphenyl borate or ammonium reineckate. The resulting precipitates were filtered, washed with cold water, allowed to dry at room temperature and grounded to fine powder.

In two glass petri dish (5 cm diameter), 10 mg of the previously prepared two ion association complex were mixed thoroughly with 0.35 ml of dibutylsebathete then add 0.19 gm of poly (vinyl chloride) (PVC). These mixtures were dissolved in 5 ml tetrahydrofuran (THF), cover the dishes with a filter paper and leave to stand overnight to allow slow evaporation of the solvent at room temperature forming master membrane with 0.1 mm thickness.

Sensors were assembled using a disk of an appropriate diameter (about 8 mm) were cut from the previously prepared master membranes and cemented to the flat end of PVC tubing with THF. A mixed solution consisting of equal volumes of 10^{-2} M AZT and 10^{-2} M potassium chloride was used as an internal reference solution. Ag/AgCl coated wire (3 mm diameter) was employed as an internal reference electrode. The sensors were conditioned by soaking for 24 h in a solution of 10^{-2} M of drug and stored in the same solution when not in use.

2.5.2. β-CD-Based Technique for the Preparation of PVC-Membrane Sensor (Sensor 3)

β-CD, 0.04 gm, was mixed with 0.35 ml of dibutylsebathete and with 0.01 gm of ammonium reineckate. PVC, 0.19 gm, previously dissolved in 6 ml THF was added and procedure was completed as above.

2.5.3. Effect of pH on the Electrode Response

The effect of pH on the potential values of the three electrode systems was studied over pH range 1-13 at 0.5 pH intervals by immersing electrodes in 10^{-3} and 10^{-4} M AZT solutions. The pH was gradually increased or decreased by adding aliquots of diluted sodium hydroxide or hydrochloric acid solutions, respectively. The potential obtained at each pH was recorded.

2.5.4. Linearity

The sensors were conditioned by soaking in 10^{-2} M AZT solution for 24 h. Storage was in the same solution when not in use. The conditioned electrodes were immersed in conjunction with the single junction calomel reference electrode in solutions of AZT in the range of 10^{-5}-

10^{-2} M. They were allowed to equilibrate whilst stirring and recording the e.m.f. readings within ±2 mV. The membrane sensors were washed between measurements with water. The potential-concentration profiles were plotted. The regression equations for the linear part of the curves were computed and used for subsequent determination of unknown concentrations of AZT.

2.5.5. Effect of Foreign Compounds on the Electrode Selectivity

The response of the three studied electrodes was also examined in the presence of a number of other related substances. The potentiometric selectivity coefficients ($K^{pot}_{AZT,\ I}$) were evaluated according to IUPAC guidelines using the separate solutions method [17,18] in which the potential of cell compromising the membrane electrode and a reference electrode is measured with two separate solutions, A and B where A (AZT ions) and B (interfering ion) at the same activity aA=aB. Selectivity coefficients were calculated by the separate solutions method, where potentials were measured for 10^{-3} M AZT solution and then for 10^{-3} M interferent solution, separately, then potentiometric selectivity coefficients were calculated using the following equation:

$$\log K^{Pot}_{A,B} = \frac{(EB-EA)}{S} + \left(1- \frac{ZA}{ZB}\right) \log aA$$

where $K^{Pot}_{A,B}$ is the potentiometric selectivity coefficient, S is slope of the calibration plot, aA is the activity of AZT, ZA and ZB are the charges on AZT and the interfering ion, respectively.

2.5.6. Application to Laboratory Prepared Mixtures

The membrane sensor was immersed in conjunction with the single junction calomel reference electrode in the different laboratory prepared mixtures. The membrane sensor was washed with water between measurements. The e.m.f. produced for each mixture was measured by the three proposed electrodes then the concentration of AZT was determined from the corresponding regression equation.

2.5.7. Application to Pharmaceutical Dosage Form

Aliquot portion of Azelast eye drop equivalent to 10^{-4} M was transferred into 10 ml volumetric flask and complete the volume to the mark by phosphate buffer pH 4. The e.m.f. produced by immersing the prepared electrodes in conjunction with single junction calomel reference electrode in the prepared solutions was determined then the concentration of AZT was calculated from the regression equation of the corresponding electrode.

2.5.8. Application to Plasma Samples

4.5 ml of plasma were placed into Stoppard shaking tube, and then 0.5 ml of 10^{-2} M AZT was added and shaken. The membrane sensor was immersed in conjunction with the single junction calomel reference electrode in these solutions. The membrane sensor was washed with water between measurements. The e.m.f. produced was measured by the three proposed electrodes then the concentration of AZT was determined from the corresponding regression equations.

3. RESULTS AND DISCUSSION

Azelastine hydrochloride is liable to alkaline hydrolysis where complete degradation was obtained after reflux with 2 M sodium hydroxide for 7 h.

The obtained degradant was separated by TLC on silica gel G F_{254} plates, using acetonitrile: water: glacial acetic acid (8: 2: 1, by volume) as developing solvent.

The structure of the alkaline degradant was elucidated by IR spectroscopy. Fig. 1 and 2 shows that the peak at 1651.07 cm^{-1} corresponding to the amide in the drug disappeared and new peak at 3425.58 cm^{-1} appeared corresponding to (OH) group of Carboxylic acid. The suggested scheme for alkaline degradation is shown in Fig. 3.

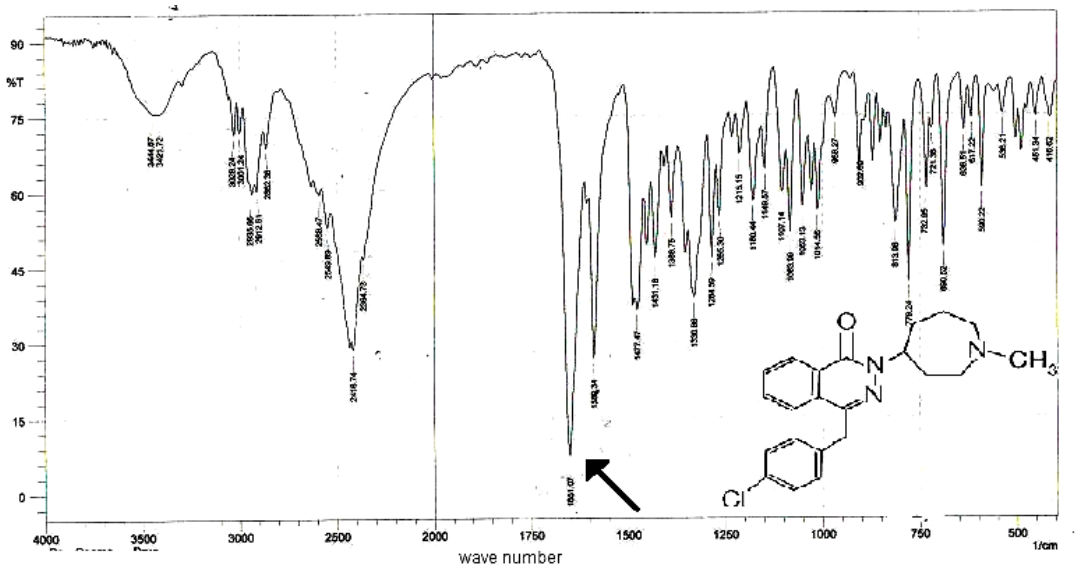

Fig. 1. IR spectrum of azelastine hydrochloride

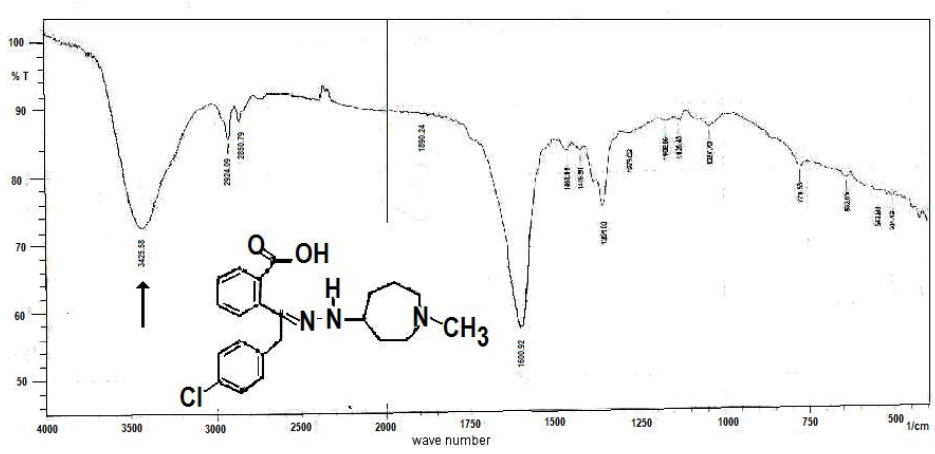

Fig. 2. IR spectrum of azelastine alkaline degradant

Reviewing the literature in hand shows that there no analytical method is reported for the determination of AZT in presence of its alkaline degrading. Therefore, the aim of this work was to develop and validate stability indicating methods for the determination of AZT in pure form and in pharmaceutical form.

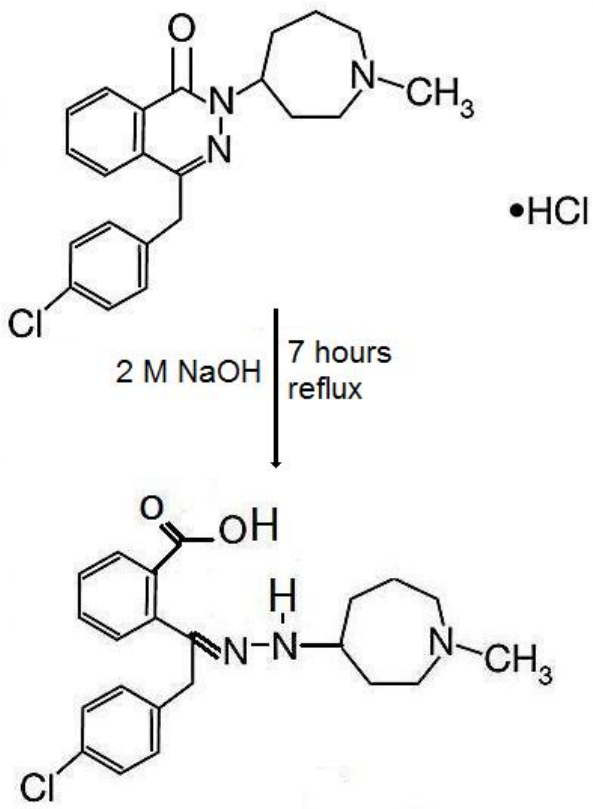

Fig. 3. Suggested scheme for the alkaline degradation of the azelastine hydrochloride

The present study originates from the fact that AZT can always act as a cation, which suggests the use of ion exchangers of the anionic type like sodium tetraphenyl borate or ammonium reineckate with the low solubility product and suitable grain size of the resulting precipitate.

AZT reacted with ammonium reineckate or Tetraphenyl borate to form stable 1:1 water insoluble ion association complex having the suggested composition shown in Fig. 4.

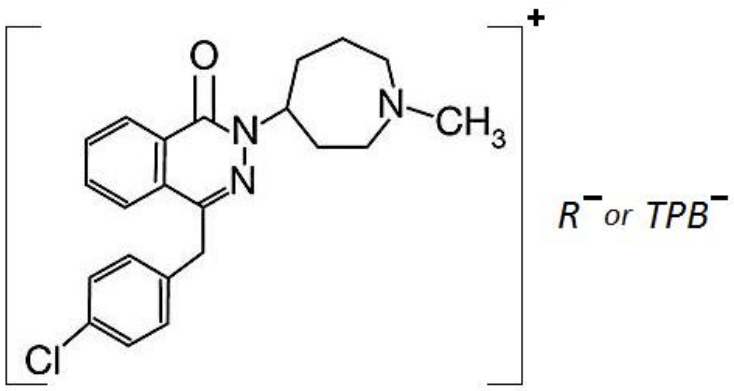

Fig. 4. suggested composition for the reaction of AZT with ammonium reineckate or Tetraphenyl borate

PVC acts as standard support matrix and as traps for the sensed ions, but its use creates a need for a plasticizer [19]. In the present investigation, dibutylsebathete was chosen as plasticizers from diesters of carboxylic acids. With PVC, the diesters of carboxylic acids were found to be the optimum plasticizers; they dissolve the ion association complex, and adjust both of the membrane permittivity and ion exchange sites mobility to give highest possible selectivity and sensitivity. Other plasticizers such as tricresyl phosphate and castor oil failed in dissolving the ion association complexes and thus gave noisy responses.

Cyclodextrins are optically active oligosaccharides that form inclusion compounds in the aqueous and in solid state with organic molecules. They were previously applied as sensor ionophores to potentiometric ISEs for the determination of protonated amines [20] and chiral molecules incorporating aryl rings [21]. β-CD-based sensors showed accurate results in both response and selectivity.

Electrochemical performance characteristics of the proposed sensors were evaluated according to the IUPAC recommendation data [17], **Table 1**. It was found that the electrodes displayed constant and stable potential readings within 2 mV from day-to-day and the calibration slopes did not change by more than 2 mV per decade over a period of 1 month for the three sensors.

Table 1. Response characteristics of the three investigated electrodes

Parameter	Sensor 1 (AZT-TPB)	Sensor 2 (AZT-R)	Sensor 3 (AZT-β-CD)
Slop (mv/decad)	-55.26	-55.59	-43.24
Intercept (mv)	220.96	200.59	180.14
Correlation coefficient	0.9996	0.9996	0.9998
Detection limit (M)	3.3×10^{-6}	6.6×10^{-6}	8.1×10^{-6}
Response time (seconds)	20-30	20-30	20-30
Working pH range	3-5	3-5	3-5
Concentration range (M)	10^{-5}-10^{-2}	10^{-5}-10^{-2}	10^{-5}-10^{-2}
Life span (weeks)	6-8	6-8	5-6
Average recovery (%)	100.01	100.09	99.94
R.S.D. %*	1.027	1.342	0.847

The response time of the electrodes were tested for concentrations of the drug from 10^{-5}-10^{-2} M. The measurements was characterized by a fast stable response within 20-30 seconds for concentrations less than 10^{-4} M and 10-20 seconds for concentrations more than 10^{-4} M.

The effect of pH on the electrode potential was investigated and it was found that the electrodes gave a useful pH range from 3-5 for sensors 1, 2 and 3 **Fig. (5-7)** above and below this pH range, the potentials displayed by the electrodes were noisy.

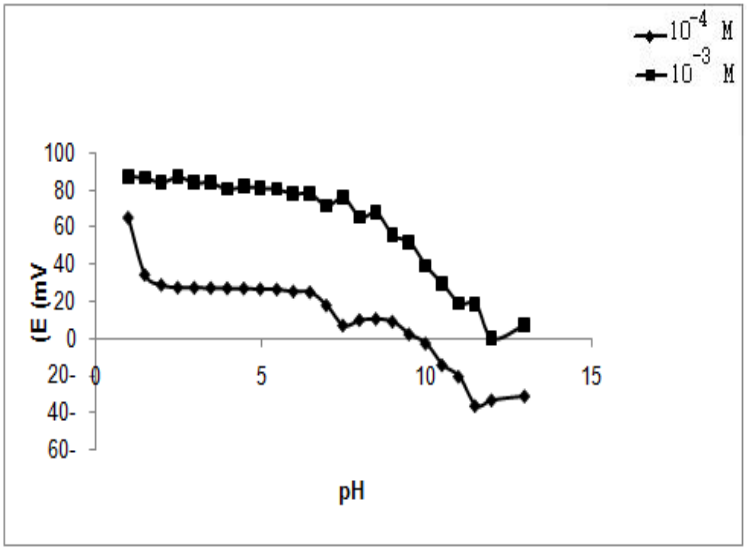

Fig. 5. Effect of pH on the response of sensor 1 (AZT-TPB)

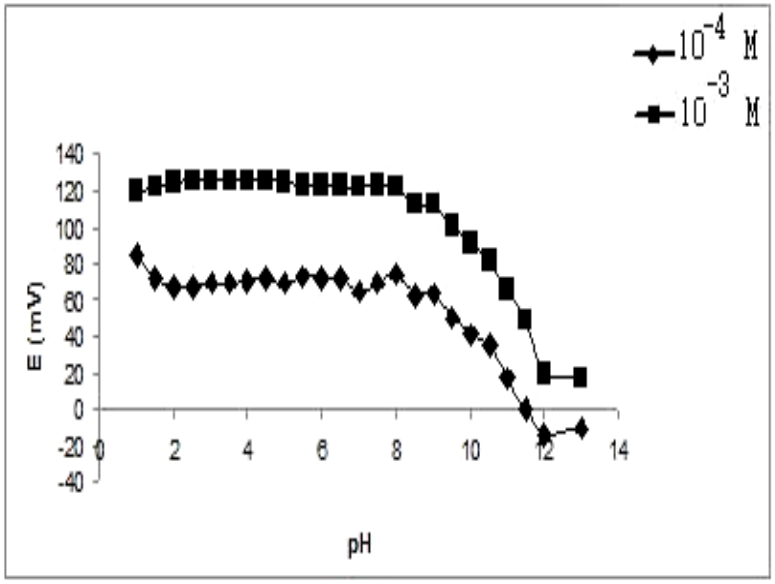

Fig. 6. Effect of pH on the response of sensor 2 (AZT- R)

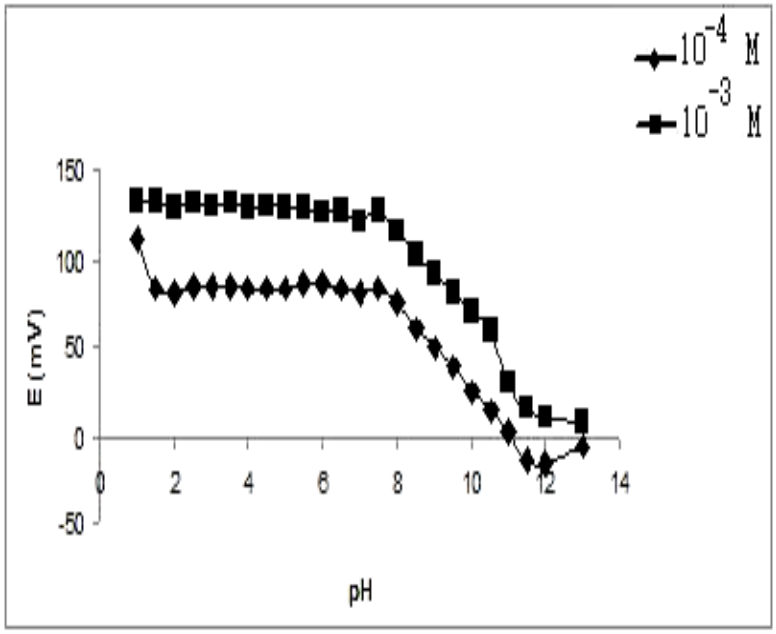

Fig. 7. Effect of pH on the response of sensor 3 (AZT- β-CD)

The potentiometric responses of the three studied electrodes at the optimum pH were linear with constant slopes over a drug concentration range 10^{-5}–10^{-2} M for sensor 1, 2 and 3, **Fig. (8-10)**.

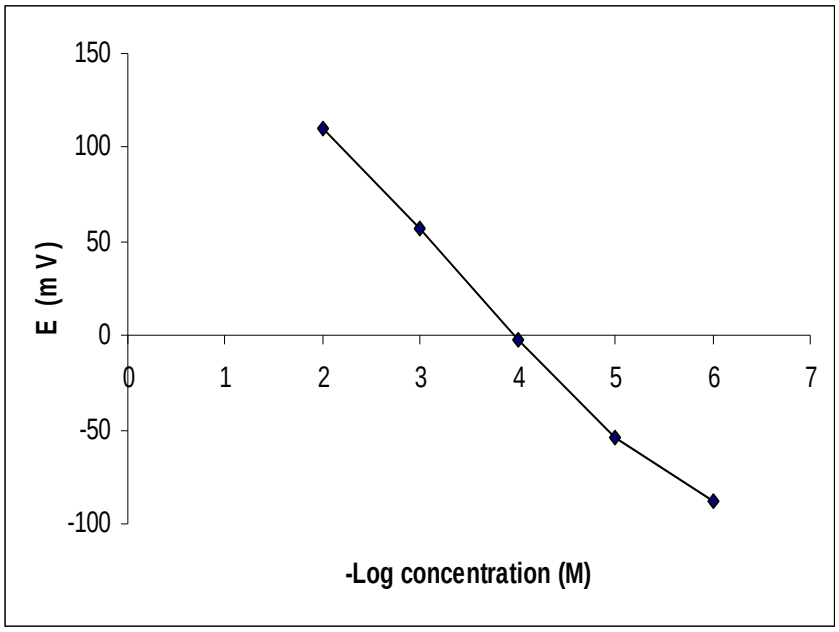

Fig. 8. Profile of the potential in mV to the -log concentration of sensor 1 (AZT-TPB)

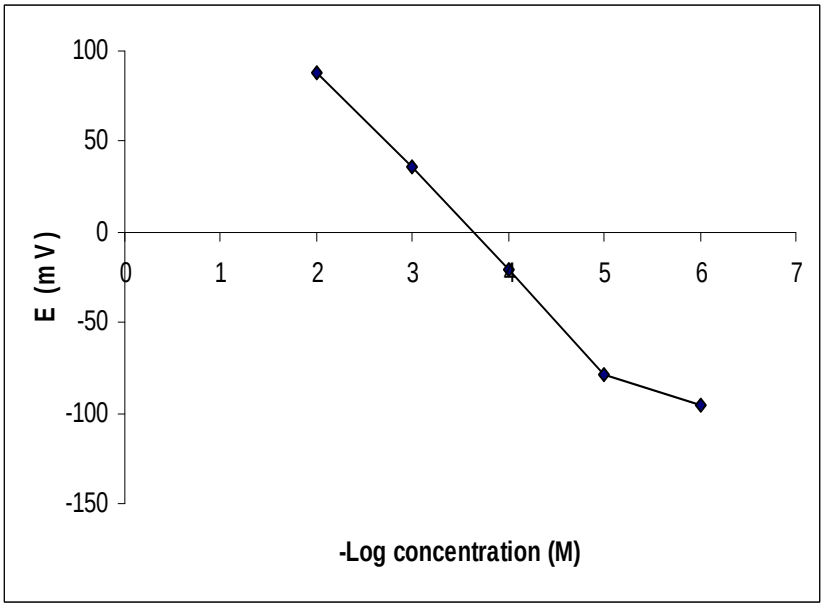

Fig. 9. Profile of the potential in mV to the -log concentration of sensor 2 (AZT-R)

Table 2. Potentiometric selectivity coefficients ($K^{pot}_{AZT, I}$) for the three proposed electrodes

Interferent	Selectivity coefficient		
	Sensor 1 (AZT-TPB)	Sensor 2 (AZT-R)	Sensor 3 (AZT- β-CD)
Degradant	13.68×10^{-3}	32.36×10^{-3}	58.88×10^{-3}
Na^{+}	1.91×10^{-3}	7.41×10^{-3}	57.54×10^{-3}
K^{+}	1.74×10^{-3}	8.71×10^{-3}	50.12×10^{-3}
NH_4^{+}	1.95×10^{-3}	9.39×10^{-3}	72.44×10^{-3}
Mg^{2+}	1.95×10^{-3}	9.24×10^{-3}	69.18×10^{-3}
Ca^{2+}	2.04×10^{-3}	8.61×10^{-3}	69.18×10^{-3}
Glycine	1.86×10^{-3}	9.24×10^{-3}	70.79×10^{-3}
Lactose	2.00×10^{-3}	10.23×10^{-3}	79.43×10^{-3}
Urea	1.86×10^{-3}	9.77×10^{-3}	74.13×10^{-3}
Glucose	2.00×10^{-3}	9.71×10^{-3}	70.79×10^{-3}
Citric acid	1.58×10^{-3}	18.62×10^{-3}	93.33×10^{-3}

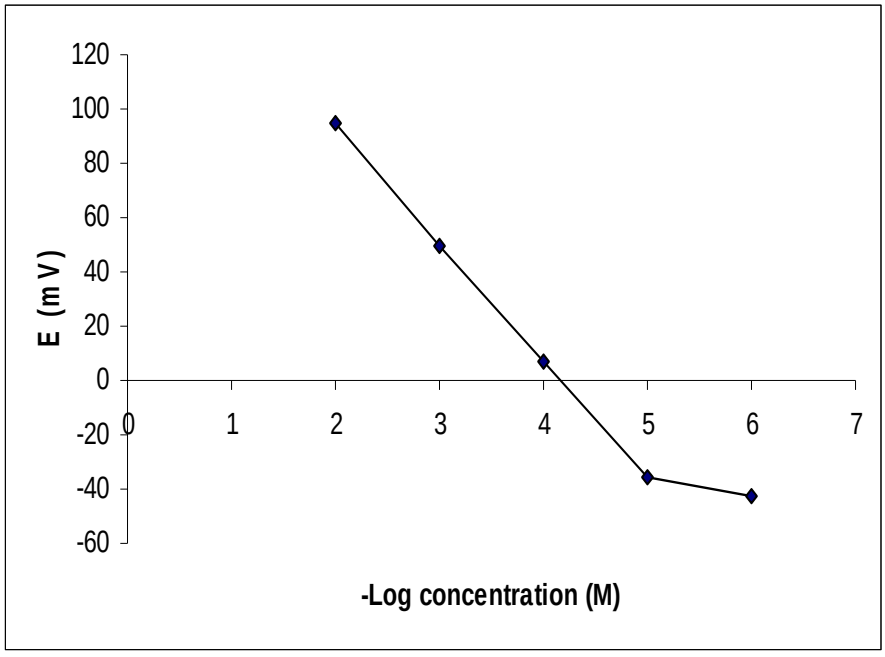

Fig. 10. Profile of the potential in mV to the -log concentration of sensor 3 (AZT- β-CD)

The accuracy of the proposed membrane sensors for the quantification of blind samples of azelastine hydrochloride was assessed by using the three sensors. The results showed average recoveries of 100.01±1.027, 100.09±1.343, and 99.94±0.846 for sensor 1, 2 and 3, respectively.

The performance of the three electrodes in the presence of alkaline degradant was assessed. Selectivity coefficient values ($K^{pot}_{AZT, I}$) were measured using a fixed concentration of the interferent (10^{-3} M). The results obtained by the developed sensors, **Table (2)**, showed reasonable selectivity of the three sensors for azelastine HCl in presence of its alkaline degradant.

Azelastine HCl was analyzed in different laboratory prepared mixtures with its alkaline degradant and good recoveries were obtained, **Table (3)**.

Table 3. Results of the analysis of AZT in different laboratory prepared mixtures with its alkaline degradant by the three electrodes

Percentage		Recovery %* of AZT		
AZT	Degradation product	Sensor 1 (AZT-TPB)	Sensor 2 (AZT-R)	Sensor 3 (AZT- β-CD)
90%	10%	100.52	100.26	100.69
70%	30%	100.40	101.32	98.15
60%	40%	100.40	98.06	99.67
50%	50%	100.83	100.66	101.80
30%	70%	100.71	98.21	99.61
20%	80%	101.55	98.56	100.67
10%	90%	101.38	97.10	99.47
Mean±SD		100.83±0.467	99.17±1.571	100.01±1.164

* Average of four determinations

Pharmaceutical additives and ingredient commonly used in drug formulation did not show any interference. Thus, analysis was carried out without prior treatment or extraction. The three sensors were successfully used for the determination of AZT in azelast eye drops, **Table 4**.

Table 4. Quantitative determination of AZT in Azelast eye drops by the proposed three electrodes

Pharmaceutical dosage form	Recovery%±S.D.* of AZT		
	Sensor 1 (AZT-TPB)	Sensor 2 (AZT-R)	Sensor 3 (AZT- β-CD)
Azelast eye drops B.No 16209	101.24 ±0.116	101.70 ±0.075	101.43 ±0.298

* Average of three determinations

On application to the biological fluids, plasma electrolyte did not show any interference. It has been found that the three electrodes gave stable results as revealed by high precision and accuracy of recoveries of the spiked plasma samples, **Table 5**.

Table 5. Determination of AZT in spiked human plasma by the proposed three electrodes

Concentration (M)	Recovery%±S.D.* of AZT		
	Sensor 1 (AZT-TPB)	Sensor 2 (AZT-R)	Sensor 3 (AZT- β-CD)
1×10^{-3}	102.71±2.132	102.32±1.981	101.21±1.543

* Average of three determinations

Statistical evaluation of the results of analysis of pure AZT by the proposed electrodes and the manufacturer method [16] showed that there is no significant difference between the proposed and manufacturer method in terms of accuracy and precision, **Table 6**.

Table 6. Statistical analysis of the results obtained by the proposed methods and the manufacturer method [16] for the analysis of AZT in pure powder form

Item	Sensor 1 (AZT-TPB)	Sensor 2 (AZT-R)	Sensor 3 (AZT- β-CD)	Manufacturer Method**[16]
Mean	100.01	100.09	99.94	99.26
SD	1.027	1.343	0.846	1.11
RSD %	1.0269	1.3418	0.8465	1.12
N	4	4	4	5
Variance	1.055	1.804	0.716	1.232
t(2.365)*	1.040	1.018	1.008	
F test	1.168 (6.26)*	1.464 (5.19)*	1.721 (6.26)*	

* The values in parentheses correspond to the theoretical values of t and F at P=0.05
** UV-spectrophotometric method

4. CONCLUSION

The three electrodes are fabricated that are sufficiently simple and selective for the quantitative determination of azelastine HCl at a wide concentration range in its pure form, in plasma and in pharmaceutical formulation in the presence of its alkaline degradant. The use of the proposed sensors offers the advantages of fast response, elimination of drug pretreatment or separation steps and low detection limit. They can therefore be used for routine analysis of azelastine HCl in quality control laboratories.

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