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Original Article

Ion Selective Electrodes with Ionophore-Doped Sensing Membranes and Graphene Nanoplatelets for Determination of Azelastine HCl in Nasal Spray and Plasma

Marwa H. Mohamed*, Neven N. Mikawy, Nancy Magdy, Amira M. El-Kosasy

Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Ain Shams University, Cairo, 11566, Egypt

ABSTRACT

Three sensors are recommended for measuring azelastine HCl (AZT) in nasal spray and spiked human plasma: the first and second sensors are calix [8]arene and hydroxypropyl beta-cyclodextrin membrane-based sensors; respectively. Both sensors consist of polyvinyl chloride (PVC) as a polymer and an electro-active agent that forms an inclusion complex with azelastine HCl. Carbon paste-based graphene nanoplatelets, a remarkable material with extraordinary mechanical, thermal, and electrical properties, constitute the third suggested sensor. The IUPAC recommendation data is used to evaluate each sensor. For the three sensors under investigation, the results provided linear responses in the concentration ranges of $1.00*10^{-2}$-$1.00*10^{-4}$, $1.00*10^{-3}$-$1.00*10^{-7}$, and $1.00*10^{-5}$-$1.00*10^{-9}$ M with Nernstian slopes of 48.37, 50.2, and 60.05 mV/decade for sensors 1, 2, and 3; respectively. A nasal spray of azelastine HCl was effectively analyzed using the three studied sensors, with mean recoveries of 101.40% ± 0.32, 100.88% ± 0.63, and 99.86% ± 0.12 for sensors 1, 2, and 3; respectively. Although good electrochemical characteristics and successful application were obtained for both sensors one and two in dosage form, the third sensor showed a better response than the first and second sensors. It has a longer lifetime (8.00-11.00 weeks) and better sensitivity, which was successfully applied for the determination of azelastine HCl in plasma with a mean recovery of 99.90% ± 0.60.

Keywords: *Azelastine HCl; calix* [8]*arene; Hydroxypropyl beta-cyclodextrin; Graphene nanoplatelets; Carbon paste.*

***Correspondence** | Marwa H. Mohamed; Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Ain Shams University, Cairo, 11566, Egypt. Email: Marwahasan.mohamed@pharma.asu.edu.eg

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1. Introduction

Azelastine 4-[(4-chlorophenyl) methyl]-2-(1-methylazepan-4-yl) phthalate-1-one hydrochloride (**Fig 1**) **[1]** was approved on 15/9/2000 as a drug used for the treatment of seasonal allergy **[2]**. Allergic rhinitis is "a disorder characterized by symptoms in the nose that are brought on by immunoglobulin-E (IgE)-mediated inflammation following exposure of the nasal membranes to allergens. IgE antibodies are produced in response to the allergen, and when they attach to mast cells, they release histamine and other inflammatory mediators **[3]**. The most common triggers are molds and pollens **[4]**. Azelastine HCl (AZT) nasal spray is available as an over-the-counter (OTC) and prescription medication. It is used to treat hay fever and other allergies (caused by an allergy to mold, dust, pollens, or pets) that result in rhinitis symptoms like runny, stuffy, or itchy noses, and itchy watery eyes **[5]**.

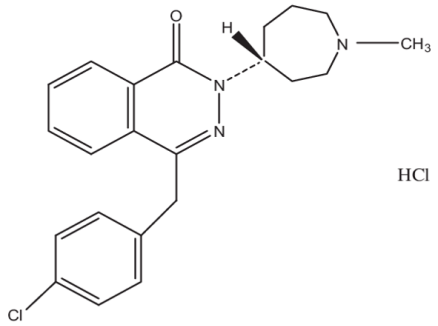

Fig. 1. AZT hydrochloride chemical structure

The methods available for the determination of AZT HCl include spectrophotometric methods **[6-9]**, spectrofluorimetric methods **[10-13]**, TLC methods **[14-16]**, HPLC **[17-26]**, NMR method **[27]**, voltammetry method **[28]**, and potentiometric methods **[29, 30]**.

Ion-selective electrodes (ISEs) are potentiometric sensors designed to reduce matrix interferences by incorporating a selective membrane **[31]**. ISEs are attractive analytical tools because of advantages such as low cost, simplicity, rapid measurements, and the fact that they do not require pretreatment of the sample **[32]**. The most common type of electrodes are polymeric membranes with ionophores **[33]**.

Ionophores are a class of molecules that combine with particular ions to produce complexes that help the ions move across cell membranes. A hydrophilic pocket (or hole) on an ionophore usually generates a binding site that is unique to that ion **[34]**. The hydrophilic pocket needs the addition of a lipophilic additive that has the opposite sign to the interest in **[35]**.

R.N. Adams employed carbon paste (CP) for the first time in electro-analysis in 1958 **[36]**. One of the most common kinds of working electrodes is the carbon paste electrode (CPE). Pastes containing insulating liquids, such as silicon oil, bromonaphthalene, tricresyl phosphate, and paraffin, account for the vast majority of CPEs used globally **[37]**. Carbon paste electrodes are highly suitable for many applications, such as the examination of traces of biological materials, organic contaminants, and metal ions **[38]**.

Graphene is a newly discovered substance with distinct chemical and physical characteristics. Recent research indicates that graphene-based composites containing diverse nanoparticles have broad potential applications in energy, environmental, and biological sciences **[39]**.

In this work, we propose two types of potentiometric sensors. The first and second sensors that are used for the determination of AZT HCl nasal spray are ionophore-based sensors that consist of polyvinyl chloride (PVC) as a polymer and electro-active agents that are based on the formation of an inclusion complex between AZT HCl and Calix [8]arene, or hydroxypropyl beta-cyclodextrin, for the first and second sensors; respectively. The third one combined the advantages of calix[8]arene and graphene nanoplatelets to enhance the analytical response and mechanical stability, which enabled us to determine AZT HCl in dosage form and human plasma.

2. Experimental

2.1. Instrumentation

Potentiometric measurements were conducted using an Ag/AgCl double-junction reference electrode (Thermo Scientific Orion 900200, (MA, and USA)) and a Jenway digital ion analyzer (model 3330; Essex, UK). 10% KNO_3 was used as the bridge electrolyte.

A Jenway pH glass electrode model 924005-BO3-Q11C (Essex, UK) was used to measure pH.

2.2. Materials and Reagents

Azelastine hydrochloride (AZT) was provided by Hikma Pharmaceutical Company

(October City, Giza, Egypt), with a purity of 99.90%.

All chemicals and reagents used were of analytical quality and water was deionized. Polyvinyl chloride (PVC), tetrahydrofuran (THF), graphene nanoplatelets (GNPs), dioctyl phthalate (DOP), ammonium molybdate, calcium chloride ($CaCl_2$), hydrochloric acid (HCl), sodium chloride (NaCl), sodium hydroxide (NaOH), magnesium sulfate ($MgSO_4$), potassium chloride (KCl), hydroxyprobyl beta cyclodextrin (β-CD**)** and Calix [8]arene were obtained from Sigma-Aldrich (Cairo, Egypt). Liquid paraffin was provided by El Nasr Company (Cairo, Egypt). Guaifenesin, paracetamol, fexofenadine, and glucose standards were provided by Amoun Pharmaceutical Company (Obour City, Cairo, Egypt), with a purity of 99.90%.

Frozen human plasma was obtained from VACSERA (Giza, Egypt).

2.2.1. Dosage form

Azelast Plus® nasal spray is labeled to contain 125.00 µg of AZT hydrochloride and 50.00 µg of fluticasone propionate (FP) per 0.137 mL.

2.2.2. Standard Solution

An AZT hydrochloride stock solution ($1.00*10^{-2}$ M) was prepared by adding 0.418 g of AZT hydrochloride to a 100.00 mL volumetric flask and completing the final volume with deionized water, then shaking well.

2.2.3. Working Standard Solutions

AZT hydrochloride stock solution ($1.00*10^{-2}$ M) was diluted with deionized water to the desired extent to prepare working solutions ($1.00*10^{-11}$-$1.00*10^{-3}$ M). The pH of each working solution was measured using a pH glass electrode; if the pH was 7.00, there was no need for any adjustment, but if it was not, a droplet of highly diluted HCl solution was used for the adjustment of pH.

2.2.4. Sensors Fabrication

2.2.4.1. Preparation of calixarene-based membrane sensor (sensor 1)

In a 5.00 cm petri dish, 0.04 g of calix [8]arene, 0.35 mL of DOP plasticizer, 0.01 g of ammonium molybdate, and 0.19 g of PVC were mixed and dissolved in 5.00 mL of THF.

The prepared solution was applied to one end of the graphite electrode surface until a soft membrane formed on it. Next, the electrode was allowed to evaporate for a full day before immersing it in AZT HCl solution ($1.00*10^{-3}$ M) for an entire night.

The body of the electrode consists of a polyethylene tube in which a graphite rod (15.00 mm in length and 5.00 mm in diameter) was inserted with a mechanically polished protruding end. The body was internally filled with liquid mercury, and a 1.00 mm copper wire was placed inside it to create an electrical connection.

2.2.4.2. Preparation of hydroxypropyl beta- β-CD-based membrane sensor (sensor 2)

In a 5.00 cm petri dish, 0.04 g of hydroxypropyl β-CD, 0.35 mL of DOP plasticizer, 0.01 g of ammonium molybdate, and 0.19 g of PVC were mixed and dissolved in 5.00 mL of THF. The same process that was stated before for the calixarene-based membrane sensor was followed.

2.2.4.3. Carbon paste sensor (sensor 3)

In a 5.00 cm petri dish, 0.30 g of GNPs, 0.04 g of calix [8]arene, 0.35 mL of DOP plasticizer, 0.01 g of ammonium molybdate, and 0.19 g of PVC were mixed, then liquid paraffin was added until it formed a paste.

To avoid any possible air gaps, with caution, the paste was placed into a 4.00 mm diameter plastic tube. To provide an electrical contact, a

1.00 mm diameter copper wire was placed inside the other end of the tube. The electrode's exterior was smoothed with soft paper. The electrode was submerged in a $1.00*10^{-3}$ M AZT HCl solution for the whole night.

2.2.5. Sensors Calibration

When the readings were stabilized, the measured potential values were recorded after each of the conditioned electrodes was submerged individually in 50.00 mL of the working standard solutions. Between readings, water was used to wash the electrodes. The measured potential values were plotted against the negative logarithmic concentrations of AZT HCl, and the regression equation for each sensor was determined using the linear section of the curve.

2.2.6. Effect of pH and temperature

Over the pH range of 1.00 to 9.00, the impact of pH on the potential values of the three electrodes was investigated at intervals of 0.50 pH by immersing the first and second electrodes in $1.00*10^{-3}$ and $1.00*10^{-4}$ M AZT HCl solutions; respectively. For the third sensor, $1.00*10^{-6}$ and $1.00*10^{-5}$ M were used.

A small amount of hydrochloric acid or sodium hydroxide solution was added to gradually raise or lower the pH. At each pH value, the potential reached was recorded.

The effect of the temperature was examined for each electrode in a temperature range of 25.00–45.00 °C by using a water bath and a thermometer to adjust the temperature of each concentration. The potential attained was recorded at each temperature for all concentrations.

2.2.7. Electrodes selectivity

The responses of the three electrodes under study were investigated in the presence of a variety of other related compounds, including inorganic cations Na^{+}, K^{+}, Mg^{+2}, and Ca^{+2}, in addition to some co-administrated drugs such as guaifenesin, fexofenadine, and paracetamol. Moreover, the study was carried out on glucose, as it is often present in human plasma.

The selectivity coefficients were calculated using the separate solution method (SSM), in which the rearranged Nicolsky-Eisenman was employed.

Log Kpot A, B = $[(E_B - E_A)/ S] + (1 - Z_A / Z_B)]$ Log [A]

Where: E_A is the electrode potential of $1.00*10^{-3}$ M AZT HCl solution for the first and second electrodes, and $1.00*10^{-5}$M for the third one, E_B is the electrode potential of $1.00*10^{-3}$ M solution of interfering ions in the first and second electrodes, and $1.00*10^{-5}$ M for the third one, S is the slope of the calibration curve for each electrode, Z_A and Z_B are charges of AZT HCl and interferent; respectively.

2.2.8. Water layer test

The water layer that may build between the electrode substrate and the ion-selective membrane may have an impact on the sensor's reaction time as well as potential drift.

In sensor 3, the test was performed by soaking the electrode in a primary ion solution ($1.00*10^{-5}$ M AZT HCl), then in a highly concentrated interfering ion solution ($1.00*10^{-4}$ M of fexofenadine), and finally back to the primary ion solution ($1.00*10^{-5}$ M AZT HCl).

2.2.9. Potential drift

To investigate the potential stability of sensor 3, the sensor was immersed in $1.00*10^{-5}$ M AZT hydrochloride solution for 1.00 h, and the potential values were recorded.

2.25.10. Application to Dosage form and spiked human plasma

2.2.10.1. Determination of AZT hydrochloride

in Azelast Plus® nasal spray

To prepare various dosage form concentrations, different aliquots were transferred from the dosage form to a 25.00 mL volumetric flask, which was subsequently completed to the final volume with deionized water. The concentrations performed were $1.00*10^{-3}$, $1.10*10^{-3}$, and $1.00*10^{-4}$ M for sensor 1, $1.00*10^{-3}$, $1.00*10^{-4}$, and $1.00*10^{-5}$ M for sensor 2, and $1.00*10^{-5}$, $1.00*10^{-6}$, and $1.00*10^{-7}$ M for sensor 3. Each of the three electrodes was then submerged in the prepared solutions, and potentials were recorded. Regression equations were used to determine concentrations of AZT HCl.

2.2.10.2. Determination of AZT hydrochloride in spiked human plasma

Various AZT HCl volumes were put into a 10.00-milliliter volumetric flask, which was subsequently filled with plasma to prepare concentrations of $1.00*10^{-8}$, $2.60*10^{-9}$, and $1.00*10^{-9}$ M. Sensor 3 was submerged in the prepared samples. Water was used to clean the membrane sensor in between measurements. After measuring the potential, the associated regression equations were used to calculate the concentration of AZT HCl and the recovery percentage.

3. Results and discussion

In this work, we make our membranes by dissolving the PVC polymer and other ingredients in an organic solvent, like THF.PVC is the most used polymer due to its favorable mechanical characteristics, low cost, and plasticization-friendliness. Although PVC functions as a typical support matrix and traps the detected ions, its utilization necessitates the addition of a plasticizer. Selecting a plasticizer is an important step. It should be a water-immiscible organic solvent with a high boiling point that plasticizes the membranes, such as DOP, dibutyl phthalate (DBP), and nitrobenzene (NB) **[40]**. DOP has good stability, less toxicity, and a suitable dielectric constant for the studied drug **[41]**. Due to the previously mentioned properties, DOP was used instead of DBP and NB.

We chose calixarenes as they have a three-dimensional cavity and variable size that can accommodate guest molecules inside their cavity. Because AZT HCl acts as a cation, calixarenes can trap it using noncovalent interactions **[42, 43]**.

In our method, we chose hydroxypropyl β-CD rather than β-CD because hydroxypropyl β-CD can trap our drug through van der Waals forces, hydrogen bonds, and hydrophobic interactions, while β-CD can trap guest molecules through hydrogen bonds only **[44]**.

Since AZT HCl can always function as a cation, the current study is based on this fact. Ammonium molybdate was added to the PVC polymer to act as an ion additive to enhance the selectivity of the membrane towards target ions (**Fig 2**).

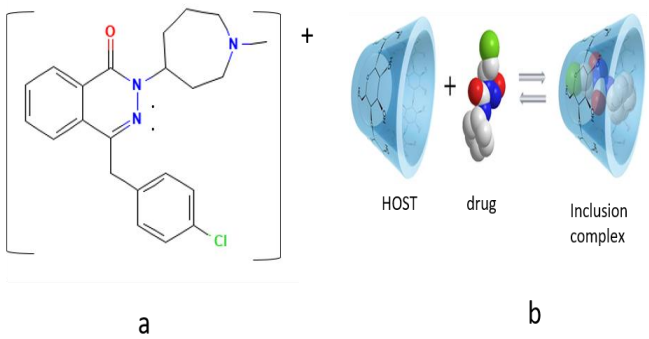

Fig. 2. Chemical Structure of (a) azelastine showing its cationic site, and (b) showing inclusion complex between drug and ionophore

A CP is made of GNPs instead of graphite powders, as GNPs exhibit exciting properties such as electrical and thermal conductivity and low cost **[45]**.

3.1. Performance characteristics of PVC-based membrane and carbon paste sensors

As the pKa of AZT HCl is 9.54 **[46]**, it will be ionized at a pH less than 7.50. In our work, we used deionized water as a solvent, and AZT HCl in the aqueous solution has a pH of 6.80 ± 0.30, so the working solutions were within the pH range of our study.

The fabricated sensors were based on the stable host-guest complex formed between the drug cation and the selected ionophores (calix [8]arene or hydroxypropyl β-CD). AZT HCl contains a phthalazinine ring that is converted to cation at a pH less than 7.80. Ammonium molybdate acts as a suitable fixed side that enhances membrane electricity.

The IUPAC recommendation data was used to assess the electrochemical performance of the suggested sensors, and the results are shown in (**Table 1**).

Table 1. General characteristics of the proposed sensors

	Sensor 1	Sensor 2	Sensor 3
Slope (mV/decade)	48.37	50.2	60.05
Intercept (mV)	455.91	1532.4	1720.6
LOD (M)	$8.17*10^{-5}$	$9.50*10^{-8}$	$5.90*10^{-10}$
Response time (seconds)	15.00	25.00	5.00
Working pH range	3.00-7.00	3.00-7.00	3.00-7.00
Concentration range (M)	$1.00*10^{-2}$ -$1.00*10^{-4}$	$1.00*10^{-3}$ -$1.00*10^{-7}$	$1.00*10^{-5}$ -$1.00*10^{-9}$
Stability (weeks)	6.00-8.00	6.00-8.00	8.00-11.00
Average recovery (mean ± SD)	100.67±0.66	100.31±0.86	99.65±0.17
Correlation coefficient	0.9997	0.9998	1
Intra-assay precision* (%RSD)	0.52	0.61	0.09
Intermediate precision* (%RSD)	0.14	0.47	0.33

*Average of three determinates

For sensors 1, 2, and 3, the calibration plots showed slopes of 48.37, 50.20, and 60.05 mV/decade; respectively, those values closely mimic the Nernstian slope of monovalent cation and agree with the reported method for determination of AZT HCl **[29]**. The linearity

ranges were $1.00*10^{-2}$ -$1.00*10^{-4}$, $1.00*10^{-3}$ -$1.00*10^{-7}$, and $1.00*10^{-5}$ -$1.00*10^{-9}$ M for sensors 1, 2, and 3; respectively (**Fig 3**).

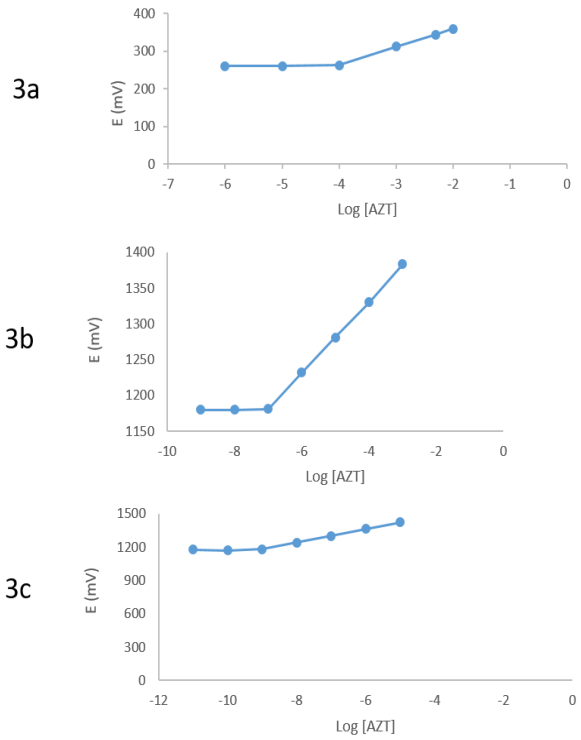

Fig. 3. Calibration curves for the three sensors (a) sensor 1, (b) sensor 2, and (c) sensor 3

The calibration graphs' extended linear segments were used to compute the limits of detection. Response time is important because it makes it possible to examine a large number of samples quickly. The findings for sensors 1, 2, and 3 were 15.00, 25.00, and 5.00 seconds; respectively. For sensors 1, 2, and 3, the corresponding lifetimes are 8.00, 8.00, and 11.00 weeks; respectively (**Fig 4**). The addition of GNPs with Calix [8]arene in sensor 3 gives the advantage of having more sensitivity, a rapid response time, a lower limit of detection, higher selectivity, and a longer lifetime. GNPs showed more stability and sensitivity to the electrical signal due to their excellent electronic and chemical properties. Moreover, the fast ion-to-electron transduction allowed short response times **[45]**.

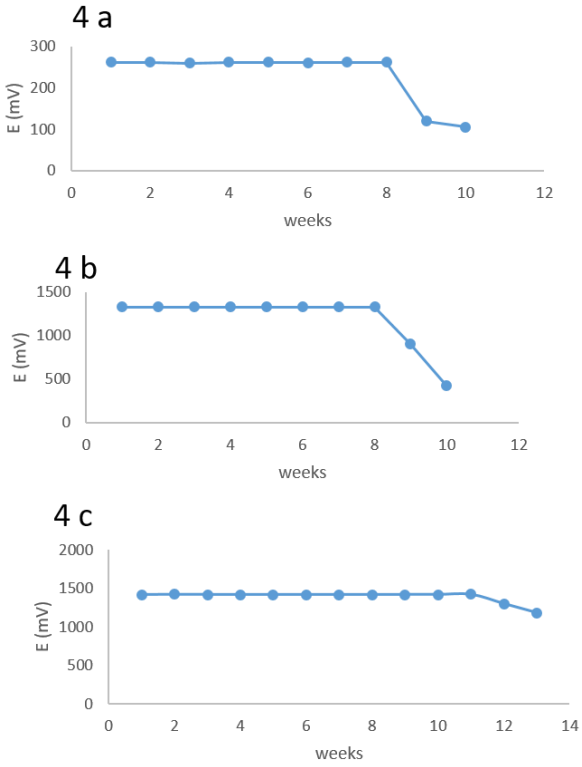

Fig. 4. Lifetime for the three sensors (a) sensor 1, (b) sensor 2, and (c) sensor 3

3.2. Effect of pH and temperature

After examining how pH affected the electrodes' potential, it was found that the electrodes obtained a suitable pH range of 3–7 for sensors 1, 2, and 3 (**Fig 5**). However, the electrodes' potentials were noisy above and below this range.

The potential variations that are seen at both lower and higher pH values are caused by the degradation of AZT HCl in highly acidic and highly alkaline mediums.

After studying the impact of temperature, it was found that sensor characteristics were stable from 25.00 to 35.00 °C. Above temperature 35.00 °C noisy results were obtained with unstable readings.

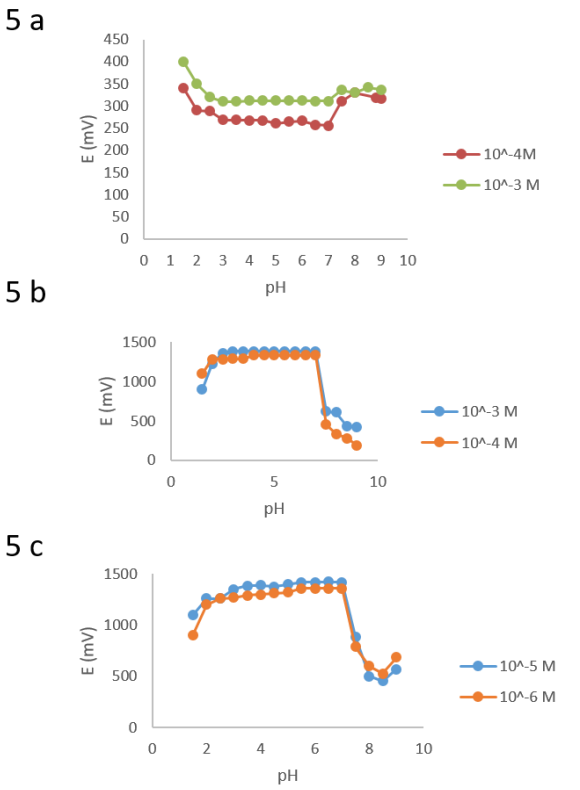

Fig. 5. Effect of pH for the three sensors (a) sensor 1, (b) sensor 2, and (c) sensor 3

3.3. Electrodes selectivity

Results summarized in **Table 2** showed the highest selectivity of the three sensors towards target ions. The three sensors were effectively utilized to determine AZT HCl without any pretreatment.

Sensor 1, which contains calix [8]arene in the membrane, showed lower values of selectivity coefficient than Sensor 2, which contains hydroxypropyl β-CD (**Table 2**). As the Calix [8]arene had a larger internal cavity size than β-CD **[47]**, this allowed the drug to fit well in the Calix [8]arene cavity and strongly bond to the Calix [8]arene. Our target in sensor 3 is to determine AZT HCl in human plasma, so we used calix [8]arene instead of hydroxypropyl β-CD due to its higher selectivity towards AZT HCl.

Comparing the selectivity of the three sensors, sensor 3 showed high selectivity (**Table 2**). Due to the addition of calix [8]arene.

Table 2. Potentiometric selectivity coefficients (Log $K^{pot}_{AZT, Interferent}$) for the proposed sensors

Interference	Sensor1	Sensor2	Sensor 3
Na^{+}	-2.15	-1.06	-2.58
K^{+}	-4.27	-3.12	-6.48
Ca^{+2}	-2.5	-1.14	-2.89
Mg^{+2}	-2.08	-2.12	-3.31
Glucose	-4.40	-2.32	-5.17
Fexofenadine	-2.58	-1.14	-2.51
Guaifenesin	-2.60	-1.10	-4.08
paracetamol	-2.39	-1.12	-4.74

3.4. Water layer test

There was not any noticeable potential drift in the sensor 3, as shown in (**Fig 6**). GNPs, which are added to sensor 3, have hydrophobic behavior that avoids the formation of water layers at the electrode/membrane interface **[45]**.

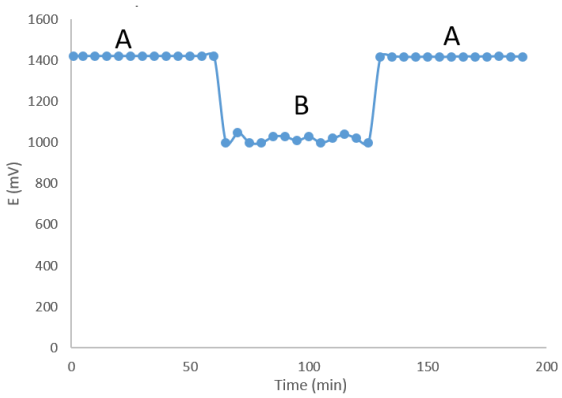

Fig. 6. Water layer test for sensor (3). Measurements were recorded in 1.00*10-5 M AZT HCl (A) and 1.00*10-4 M of fexofenadine (B)

3.5. Potential drift

Potential measurements showed null change with small drifting (0.50 mV/h) and a rapid response time of 5.00 seconds (**Fig 7**). The stability of the electrode potential is due to the addition of GNPs that allow the transfer of ion electrons through the membrane.

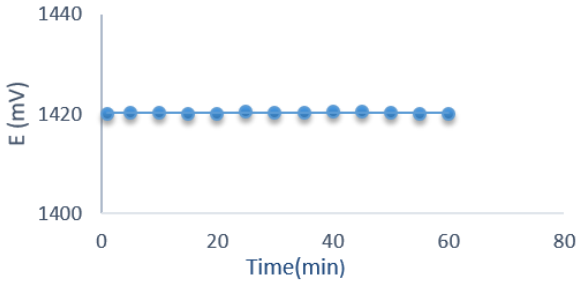

Fig. 7. Potential drift for sensor (3). Measurements were recorded in $1.00*10^{-5}$ M AZT HCl

3.6. Determination of AZT hydrochloride in Azelast Plus® nasal spray

As the three sensors are selective to AZT HCl in the presence of other components in dosage form, they can be used for the determination of AZT HCl. **Table 3** shows that all sensors achieved good recoveries.

Table 3. Recovery results of three sensors for determination of AZT hydrochloride in Azelast Plus® nasal spray

Sensor 1			Sensor 2			Sensor 3		
Claimed conc (M)	**Found* conc (M)**	**%Recovery**	**Claimed conc (M)**	**Found* conc (M)**	**%Recovery**	**Claimed conc (M)**	**Found* conc (M)**	**%Recovery**
$1.10*10^{-3}$	$1.12*10^{-3}$	101.81	$1.00*10^{-3}$	$1.01*10^{-3}$	101.33	$1.00*10^{-5}$	$9.98*10^{-6}$	99.83
$1.00*10^{-3}$	$1.01*10^{-3}$	101.33	$1.00*10^{-4}$	$1.02*10^{-4}$	101.25	$1.00*10^{-6}$	$9.98*10^{-7}$	99.83
$1.00*10^{-4}$	$1.02*10^{-4}$	101.75	$1.00*10^{-5}$	$1.01*10^{-5}$	100.20	$1.00*10^{-7}$	$1.00*10^{-7}$	100.00
Mean ± SD	101.63 ± 0.38		**Mean ± SD**	100.93 ± 0.63		**Mean ± SD**	99.86 ± 0.12	

*Average of three determinates

3.7. Determination of AZT hydrochloride in spiked human plasma

The human plasma concentration (Cmax) of the drug in human plasma equals 1.10 ng/mL **[48]**, which is equivalent to $2.60*10^{-9}$ M. Sensor 3 can only be used for the determination of AZT HCl, as it includes that concentration in its concentration range. Recovery results were acceptable, as shown in **Table 4**.

Table 4. Recovery results of carbon paste sensor for determination of AZT hydrochloride in spiked human plasma

	Sensor 3	
Claimed conc (M)	**Found conc* (M)**	**%recovery**
$1.00*10^{-8}$	$9.92*10^{-9}$	99.25
$2.60*10^{-9}$	$2.61*10^{-9}$	100.46
$1.00*10^{-9}$	$1.00*10^{-9}$	100.00
Mean ± SD	99.90 ± 0.61	

*Average of three determinates

3.8. Comparison between reported methods and our proposed method

There are two potentiometric methods reported for the determination of AZT HCl in different dosage forms and human plasma. The first method **[29]** used the precipitation technique and β-CD technique for the preparation of PVC membranes and used them for the determination of AZT HCl in eye dosage form and human plasma. The second method **[30]** used the precipitation technique for the preparation of the PVC membranes and applied them for the determination of AZT HCl in nasal spray dosage forms. In our method, we used Calix [8]arene and hydroxypropyl β-CD for the preparation of PVC membranes, and we used GNPs coupled with Calix [8]arene for the preparation of carbon paste sensor. As shown in **Table 5**, the use of hydroxypropyl β-CD instead of β-CD gave us a wider concentration range than β-CD. Our sensors have a faster response time, a longer lifetime, a lower limit of quantification, and more concentration ranges in comparison with other reported methods. For the first time, the concentration range of sensor 3 includes the Cmax of AZT HCl, which allowed us to determine AZT HCl in human plasma (**Table 5**).

3.9. Statistical analysis

The suggested technique was effectively used to accurately and precisely determine levels of AZT HCl in nasal spray and spiked human plasma. According to statistical compression, there is no significant difference between the published approach **[27]** and our suggested way because the *t*-test and F-test are less than the tabulated ones, as shown in **Table 6.**

Table 5. Comparison between reported potentiometric methods and proposed method for analysis of AZT hydrochloride

Items	Reported method[29]			Reported method[30]	Proposed method		
	Sodium tetraphenylborate as a precipitating agent	Ammonium reineckate as a precipitating agent	β-CD-Based Technique	Tetrafluorophenyl borate as a precipitating agent	Sensor 1	Sensor 2	Sensor 3
Detection limit (M)	$3.3*10^{-6}$	$6.6*10^{-6}$	$8.1*10^{-6}$	$1*10^{-5}$	$8.17*10^{-5}$	$9.5*10^{-8}$	$5.9*10^{-10}$
Response time (seconds)	20-30	20-30	20-30	8	15	25	5
Concentration range (M)	10^{-5}-10^{-2}	10^{-5}-10^{-2}	10^{-5}-10^{-2}	$1.8 * 10^{-5} – 10^{-1}$	10^{-2} -10^{-4}	10^{-3} -10^{-7}	10^{-5} -10^{-9}
Life span (weeks)	6-8	6-8	5-6	3	6-8	6-8	8-11

Table 6. Statistical comparison of the results obtained by the proposed method and the reported method for analysis of AZT hydrochloride

Items	Reported method	Proposed method		
		Sensor 1	Sensor 2	Sensor 3
Mean	100.56	100.67	100.31	99.65
SD	0.59	0.66	0.86	0.17
Variance	0.35	0.44	1.99	0.03
n	3	4	4	4
t		0.21 (2.57)*	2.02 (2.57)*	2.56 (2.78)*
F		1.25 (19.16)*	1.38 (19.16)*	2.00 (19.0)*

*The values between parentheses are the corresponding theoretical values of t and F at the 95%confidence level

Conclusion

In this work, we proposed three simple, fast, and novel sensors for the determination of AZT HCl in the nasal spray dosage form. The first and second are ionophore-based sensors that are based on the formation of an inclusion complex between AZT HCl and Calix [8] arene, or hydroxypropyl β-CD, for the first and second sensors; respectively. The third one is the carbon paste sensor. The third sensor exhibits a faster response time, and a longer lifetime, and is more selective towards target ions than previous sensors because of the benefit of combining GNPs with Calix [8]arene. Adding GNPs increases the sensitivity of the electrode, which allows us to successfully determine AZT HCl in

human plasma.

Declarations

Ethics Approval and Consent to Participate

Not applicable (As the work is not in real samples or clinical trials so no need for ethical approval for the work).

Consent to Publish

All authors have read and agreed to the published version of the manuscript.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article in the main manuscript.

Competing Interests

The authors declare that no competing interests exist.

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Authors' Contributions

The manuscript was drafted and written by Marwa Hassan Mohamed

All authors have read and approved the final - manuscript

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