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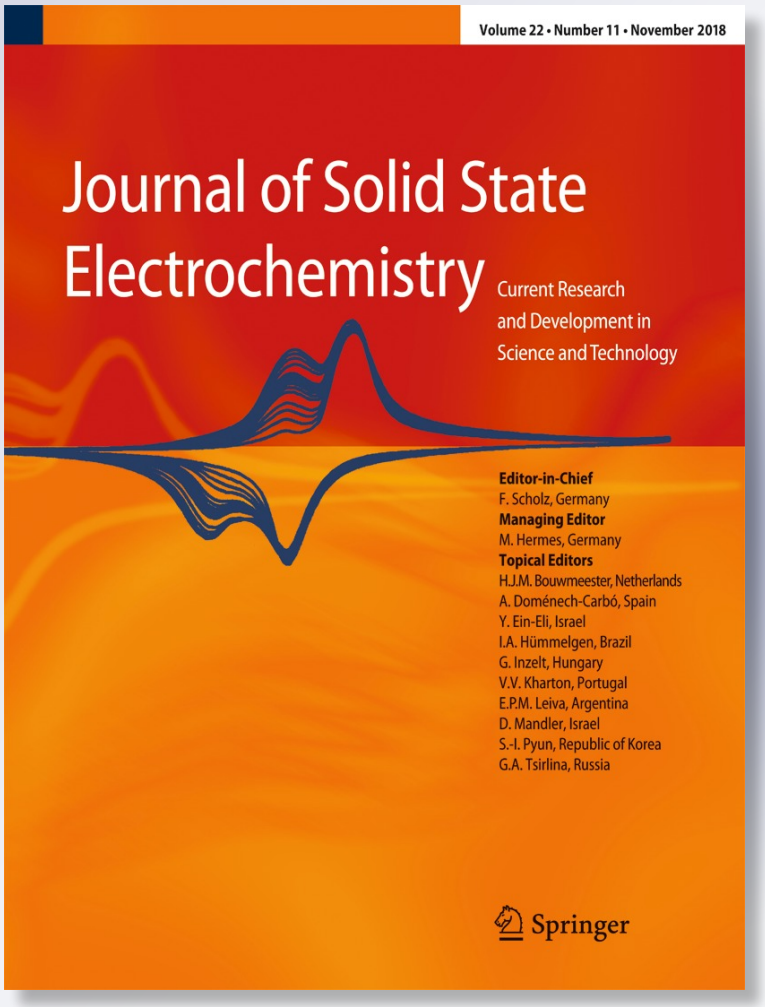

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ORIGINAL PAPER

Potentiometric ion-selective electrodes for determination of cyclopentolate hydrochloride and phenylephrine hydrochloride in their challenging ophthalmic formulation

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Abstract

Introduction of potentiometric ion-selective electrodes (ISEs) opened a new bright area in pharmaceutical analysis acknowledged as being an eco-friendly, simple, and energy-saving technique that is well-suited with microfabrication. In this contribution, potentiometric ISEs were employed as an alternative green analytical tool with the crucial goal of expanding the effective application of the potentiometric sensors in different disciplines of drug-stability studies and quality-control investigations. Four novel cyclopentolate hydrochloride and phenylephrine hydrochloride selective membrane sensors were constructed and evaluated. Sensors' fabrication was achieved using potassium tetrakis (4-chlorophenyl) borate, a cationic exchanger, in a polyvinyl chloride polymeric matrix plasticized with 2-nitrophenyl octyl ether and using 2-hydroxy propyl-β-cyclodextrin as an ionophore. A comparative potentiometric study was implemented using two designs of ISEs; a conventional liquid inner contact and a glassy carbon solid-contact one. Using solid-contact ISEs, detection limit was substantially decreased and the discriminative ability in the presence of the most interfering substances was enhanced. This permits simultaneous estimation of both drugs, in spite of their similar ionic characteristics, abolishing the need for any pretreatment or separation steps in their challenging combined ophthalmic formulation as well as in rabbit aqueous humor and in the presence of their degradation products.

Keywords Cyclopentolate hydrochloride · Phenylephrine hydrochloride · Green analytical chemistry · Liquid inner contact electrode · Solid-contact electrode · Stability-indicating method · Rabbit aqueous humor

Introduction

An eco-friendly approach in pharmaceutical analysis is the key driver for moving towards green analytical methods (GAM) aiming to sustainable development and environment preservation. Nowadays, efforts are directed towards employing the green analytical chemistry (GAC) principles to reduce the negative influence of different analytical methodologies. Minimizing waste generation, analysis time, risky solvent consumption, sample size, and energy saving are the main goals for development of GAM. Remarkably, modern electroanalytical chemistry is up to the contest of investigating new tactics fulfilling the requirements of GAC with great prospect to solve challenging analytical tasks [1]. The innovative potentiometric ion-selective electrodes (ISEs) could provide beneficial tools characterized by not only being eco-friendly, but also relatively simple, rapid, non-destructive, and adaptable to small sample volume.

Cyclopentolate hydrochloride (CLO) is chemically 2-(dimethylamino)ethyl (*2RS*)-(1-hydroxy cyclopentyl) (phenyl)acetate hydrochloride) [2]. It is used in ophthalmic diagnostic procedures to produce mydriasis and cycloplegia [3]. Phenylephrine hydrochloride (PHE) is chemically (1*R*)-1-(3-hydroxyphenyl)-2-(methylamino)ethanol hydrochloride) [2]. It is a mydriatic agent in ophthalmology [3]. Both CLO and PHE are commonly co-formulated as ophthalmic solution. Their chemical structures are shown in Fig. 1. Considerable attention has been paid towards studying the

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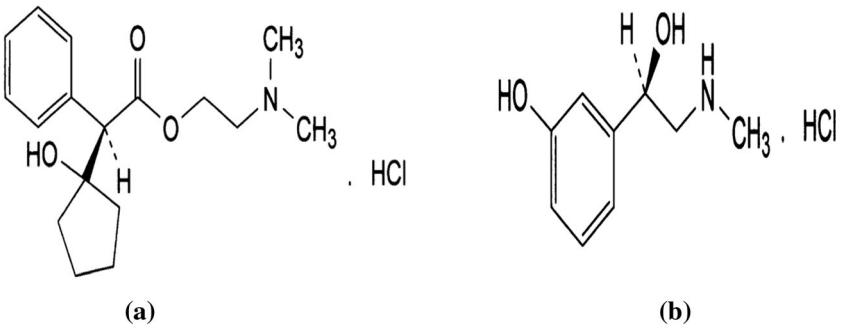

(a) (b)

Fig. 1 Chemical structure of **a** cyclopentolate HCl and **b** phenylephrine HCl

chemical stability of CLO and PHE particularly in drug preparations using different analytical techniques. A comprehensive review of literature indicates dominance of separation-based chromatographic methodologies, as stability-indicating assays; for tracking the degradation products [4–7]. However, these techniques comprise various complicated difficulties, such as multiple sample extraction steps, prolonged analysis times, and expensive instruments, and they are unsuitable to colored and turbid solutions, in addition to employing environmentally unfriendly hazardous organic solvents. Few ion-selective electrodes have been reported for estimation of CLO [8] or PHE [9–11] using a precipitation-based technique with different cation exchangers without ionophores. However, these electrodes did not examine the chemical stability of CLO and PHE or the selective measurement in their combined pharmaceutical formulation without the need for pretreatment or separation step. Taking all these into account, we addressed the first green approach that involves the design, study, and then comparison of novel potentiometric sensors for the selective determination of both drugs under study, in spite of their similar ionic characteristics, that represent a challenging task that we directed our research to solve.

Both liquid and solid-contact ion-selective electrodes (SC-ISEs) have been successfully employed in different disciplines of drug-related analytical investigations. However, SC-ISEs exceled over the conventional liquid inner-contact ones that suffer from some limitations such as being restricted to vertical measurements, difficult to miniaturize, ion leakage from the internal solution, and unsuitability to operate under high pressure due to the presence of inner filling solution. Attractively, SC-ISEs could provide a promising substitute with better response features. They offer rigid and robust sensors with good miniaturizing capability and enhanced long-term stability [12, 13].

The present contribution was motivated by not only further exploring how potentiometry could be used as a green alternative analytical tool closely adhering to GAC principles but also to investigate the opportunities and challenges offered by SC-ISEs in respect to the corresponding conventional liquid inner-contact ones. In this work, two new electrodes of different integration shapes were designed, a liquid inner contact (electrode A) and a glassy carbon solid-contact one (electrode B). This study critically highlights the benefits and limitations offered by the two different ISEs assemblies regarding detection limits, concentration ranges, and the effect of the internal solution. Four novel sensors were introduced using a PVC membrane doped with 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD) as an ionophore. Initially, the performance characteristics of the proposed sensors for selective estimation of CLO (sensors 1 and 3) and PHE (sensors 2 and 4) were evaluated to select the optimum working conditions. Subsequently, a stability-indicating study was performed. Finally, to endorse the inferences above, the proposed sensors were implemented to determine CLO and PHE, without prior separation, in different pharmaceutical and biological samples.

Experimental

Instruments

The external reference electrode was a Thermo Scientific Orion double junction Ag/AgCl reference electrode (No. 900200, MA, USA) with 3.0 mol L^{-1} KCl saturated with AgCl as an inner filling solution and 10% KNO_3 as bridge electrolyte. Potentiometric measurements were performed using Jenway digital ion analyzer (model 3505; Essex, UK) in a magnetically stirred solutions using Bandelin Sonorox, Rx510S (Budapest, Hungary) magnetic stirrer. pH adjustments were carried out employing Jenway pH glass electrode (Essex, UK). Glassy carbon electrode was used as the solid contact, 3-mm diameter, CH Instruments (Texas, USA).

Samples

Pure standard

Cyclopentolate hydrochloride and phenylephrine hydrochloride were kindly obtained from the Kahira Pharmaceuticals and Chemical Industries Co. (Cairo, Egypt), with potency of 100.10 ± 1.32% and 99.81 ± 1.07%, respectively; according to BP official methods [2].

Pharmaceutical formulations

Cyclophrine® eye drops, BN 1560308, manufactured by Kahira Pharmaceuticals and Chemical Industries Co. (Cairo, Egypt). Each 5 mL was claimed to contain 50 mg CLO and 500 mg PHE in a sterile aqueous buffered solution.

Colircusi Cicloplejico® eye drops, BN 5L CG2A, were manufactured by Alcon Cusi, S.A., El Masnou, Barcelona, Spain. Each milliliter was labeled to contain 10 mg of CLO in a sterile aqueous buffered solution.

Degraded samples

Preparation of CLO alkali-induced degradation products A mass of 10.0 mg of CLO was dissolved in 10 mL of 0.1 mol L^{-1} sodium hydroxide and allowed to stand at ambient temperature for 2 h. The degraded sample was then neutralized, transferred into a 25-mL volumetric flask and brought to volume with deionized water.

Preparation of PHE oxidative degradation products Oxidative degradation was done by refluxing a mass of 10.0 mg of PHE with 20 mL of 3% H_2O_2, for 3 h. The solution was then transferred into a 25-mL volumetric flask. The volume was completed with deionized water.

Complete degradation was tested for both drugs by HPTLC method, as reported previously [7].

Chemicals and reagents

Analytical-grade chemicals, reagents, and bi-distilled deionized water were used. Polyvinyl chloride (PVC) and 2-nitrophenyl octyl ether (NPOE) were obtained from Fluka Chemie GmbH (Steinheim, Germany). Potassium tetrakis (4-chlorophenyl) borate (TpClPB), (2-Hydroxypropyl)-β-cyclodextrin (2-HP-β-CD), tetrahydrofuran (THF), potassium dihydrogen orthophosphate, sodium hydroxide, and hydrochloric acid were purchased from Sigma-Aldrich (Steinheim, Germany). Potassium chloride was obtained from Fluka AG (Buchs, SG, Switzerland).

Standard solutions

Stock standard solutions

CLO and PHE stock standard solutions (1.0×10^{-2} mol L^{-1}) were prepared by accurately transferring 0.328 and 0.204 g of pure CLO and PHE, respectively, into two separate 100-mL volumetric flasks, then dissolved in phosphate buffer pH 4.0 ± 0.2. The volume was completed to mark with the same solvent. The stability of the prepared solutions was assessed for at least 24 h at 25 °C and all solutions were protected from light.

CLO alkali-induced degradation products and PHE oxidative degradation products equivalent to 1.0×10^{-3} mol L^{-1} were prepared by accurately transferring 20.50 and 12.80 mL of CLO and PHE corresponding degradation products stock solutions (0.4 mg mL^{-1}), respectively, into two separate 25-mL measuring flasks and the volumes were diluted to mark with phosphate buffer, pH 4.0 ± 0.2.

Working standard solutions

CLO and PHE working standard solutions (1.0×10^{-7}–1.0×10^{-3} mol L^{-1}) were freshly prepared by making suitable dilutions from their respective stock standard solutions, using phosphate buffer pH 4.0 ± 0.2.

Procedure

Sensor fabrication and calibration

Preparation of conventional liquid inner-contact, electrode A (sensors 1 and 2): a mass of 10 mg of TpClPB was thoroughly mixed with 0.35 mL of o-NPOE, 190 mg PVC, and 10 mg 2-HP-β-CD in a glass petri dish (5-cm diameter). These components were dissolved in 6 mL THF by stirring using a glass rod. Allowed for solvent evaporation overnight, two disks (about 8 mm in diameter) were cut from the parent membrane using a cork borer and were then glued with the aid of THF to a PVC tip that was clipped into the end of an electrode glass part. Equi-volume of either 1.0×10^{-2} mol L^{-1} CLO (sensor 1) or 1.0×10^{-2} mol L^{-1} PHE (sensor 2) and 1.0×10^{-2} mol L^{-1} potassium chloride (prepared in distilled water) were used as inner filling solution for electrodes conditioned for 24 h by soaking in 1.0×10^{-2} mol L^{-1} of aqueous corresponding drug solution. Ag/AgCl wire (1 mm diameter) was used as an inner reference electrode.

Fabrication of solid-contact (glassy carbon), electrode B (sensors 3 and 4): 20 μL of the previously prepared liquid ion-selective membrane cocktail in THF was directly applied using a micropipette on a glassy carbon electrode, previously polished and cleaned with water. The entire outer membranes were then allowed to evaporate overnight to produce a uniform membrane on the surface, before the solid-contact electrodes were conditioned, separately, by soaking sensor 3 in 1.0×10^{-2} mol L^{-1} CLO and sensor 4 in 1.0×10^{-2} mol L^{-1} PHE for 24 h and they were stored in the same solutions when not in use.

For the construction of calibration curves, each sensor separately, in conjunction with the reference electrode, was calibrated by being immersed in its respective drug solutions (1.0×10^{-7} to 1.0×10^{-2} mol L^{-1}) prepared in phosphate buffer solution pH 4.0 ± 0.2. Sensors were allowed to equilibrate while stirring until a constant reading of the potentiometer was reached. The potential differences (*emf*) between the

membrane sensor (indicator electrode) and the reference electrode were recorded after stabilizing to ± 0.1 mV as a function of CLO and PHE concentrations. Calibration plots were then constructed relating the recorded electrode potentials obtained by the four proposed sensors versus logarithmic molar drug concentrations of the corresponding drugs. The regression equations were then obtained for the linear responses of the calibration plots and were used for subsequent measurements of unknown samples of CLO and PHE. All sensors were washed with phosphate buffer pH 4.0 ± 0.2 before and after each run till reaching a constant potential.

Direct potentiometric determination of laboratory-prepared mixtures containing different ratios of CLO and PHE in the presence of their possible degradation products

Measurements were carried out in different synthetic mixtures containing varying concentrations of intact drugs along with their degraded samples in different ratios (including the market product ratio) in phosphate buffer pH 4.0 ± 0.2. The potential readings obtained by immersing the described sensors for both drugs in conjunction with the reference electrode in the prepared mixtures were recorded. Concentration of each drug was calculated in each mixture from its consequent regression equations.

Application to pharmaceutical formulations by the proposed potentiometric method

Two volumes of Colircusi Cicloplejico® eye drops and Cyclophrine® eye drops equivalent to 10.0 mg CLO were accurately transferred separately into two 25-mL volumetric flasks, and the volumes were diluted to the mark with phosphate buffer pH 4.0 ± 0.2. Suitable dilutions of the prepared solutions were applied using the same solvent. The obtained potential was compared to the respective calibration plots.

Application to spiked rabbit aqueous humor by the proposed potentiometric method

This work was approved by the Institutional Research Ethical Committee at Faculty of Pharmacy, Cairo University.

Seven albino rabbits, weighing 2–2.5 kg, free of any signs of ocular inflammation or any clinical observable defects were used. The rabbits were housed in well-ventilated room and fed customized meals and water. In order to gather the aqueous humor, the rabbits were locally anesthetized with 0.4% benoxinate hydrochloride solution. Two drops were drilled into rabbit's eye. Aqueous humor samples were collected by anterior chamber paracentesis using a small syringe. The procedure was repeated two times a day for about 5–7 days till the required volume was collected. Samples were stored at − 20 °C until the experiment was carried out. After removal of aqueous humor samples at each time interval, the ocular surface was wetted with isotonic phosphate buffered saline and dried with soft tissue. Spiked aqueous humor samples were prepared by transferring suitable aliquots of CLO (1.0×10^{-4} mol L^{-1}) and PHE (1.0×10^{-3} mol L^{-1}) working standard solutions or Cyclophrine eye drops® into a 10-mL volumetric flask, where the volume was completed to mark with the collected aqueous humor, and then transferred into 25-mL beakers. The beakers were vortexed for 1 min. The sensors and the reference electrode were immersed in these solutions and washed with water between measurements. The emf produced for each solution was measured by the proposed sensors, and then the concentration of CLO and PHE was determined from the corresponding regression equation.

Study of the experimental conditions

Estimation of the slope, response time, and operative life of the proposed sensors The electrochemical performance of the four proposed sensors was tested as per IUPAC recommendations [14].

Effect of pH and temperature on the electrode response The effect of pH on the potential values of the four investigated sensors was studied over pH range 2.0–8.0 by immersing the electrodes in 1.0×10^{-3} and 1.0×10^{-4} mol L^{-1} aqueous CLO and PHE standard solutions. The obtained potential at each pH value was recorded.

The potential displayed by the studied sensors was monitored as a function of temperature in the range of 25 to 35 °C. The potential obtained at each temperature was recorded.

Electrodes' selectivity The potential response of the four proposed sensors was tested in the presence of susceptible interfering species. Potentiometric selectivity coefficient, $K^{\text{Pot.}}{}_{\text{primary ion, interferent}}$, was used to evaluate the extent to which a foreign substance would interfere with the response of the electrodes to its primary ion. Selectivity was determined by separate solutions method (SSM) [14], where potentials were measured for 1.0×10^{-3} mol L^{-1} interferent solution and then for 1.0×10^{-3} mol L^{-1} respective drug solution, separately, then selectivity coefficients were calculated using the following equation:

$$-\log\left(K^{\text{pot}}{}_{\text{primary ion,interferent}}\right) = (E_1 - E_2)/S$$

where E_1 is the potential measured (mV) in 1.0×10^{-3} mol L^{-1} of their primary ion (CLO in case of sensors 1 and 3 and PHE in case of sensors 2 and 4) solution, E_2 is the potential measured in 1.0×10^{-3} mol L^{-1} of the interferent solution, and S represents the slope of the investigated sensors (mV/concentration decade).

Results and discussion

Synchronized multi-component's pharmaceutical analysis denotes a challenge due to a large number of analytes present in the sample matrix. These analytes are not only the active pharmaceutical ingredients but also the degradation products or impurities that might tail the active substances and would have significant impact on product safety and efficacy. Owing to the outstanding properties addressed by ISEs as sustainable analytical procedure, it is rewarding to develop novel fabricated electrodes with competitive characteristics for the selective estimation of CLO and PHE in their combined dosage form, without prior separation, or in the presence of their degradation products and related substances.

Considering these points, the present study was done to design and optimize the new proposed sensors: a conventional, liquid inner, contact electrode and a solid-contact, glassy carbon one of CLO and PHE, and then a comparison was addressed.

Fabrication and performance characteristics of the proposed sensors

In the present work, both CLO (p*K*a 7.93) and PHE (p*K*a 8.42) [15] behave as cations in acidic and nearly neutral medium due to the presence of tertiary and secondary amino groups, respectively. An ion-exchanger of cation exchange type; potassium tetrakis (4-chlorophenyl) borate (TpClPB) was that kind of choice in the fabrication of the proposed sensors. In situ complex formation was done as each membrane was primarily conditioned into 1.0×10^{-2} M of respective drug solution for 1 day in order to replace the original exchangeable counter ion (K^+) of the ion exchanger with either CLO or PHE. In contrast to precipitation-based technique, the lack of complex incorporation in membrane fabrication was advantageous for being more straightforward, economical, and enabled flexibility in applications since a single membrane could be used for numerous analytes.

Unlike PHE, CLO is present in low concentration in Cyclophrine® eye drops (only 50 mg versus 500 mg of PHE), thus achieving more selective and sensitive sensors was a necessity. To enhance the selectivity, 2-HP-β-CD ionophore was added based on its outstanding complexation properties. Preliminary studies were carried out to examine the selective recognition of CLO and PHE by the 2-HP-β-CD ionophore in the membrane phase; the performance of an ionophore-based ISE was compared with an ionophore-free ion-exchanger TpClPB as a control experiment. In the absence of the ionophore, the lowest slope and the highest selectivity coefficient values are found (Fig. 2). On the other hand, the 2-HP-β-CD-based sensors showed improved Nernstian slope and selectivity coefficient values (displayed latter). It should be noted that functionalized lipophilic CD derivatives, 2-HP-β-CD, form inclusion complexes or nanostructure supramolecular assemblies in their hydrophobic cavity with a variety of guest molecules; thus enhance both selectivity and sensitivity of ISEs. The results reveal that, the positive CLO and PHE ions prefer the high donation sites (OH-groups) of 2-HP-β-CD (Supporting information, Fig. S1). The host-guest complex is stabilized via intermolecular hydrogen bonding, hydrophobic interactions, and van der Waals forces [16]. Moreover, the large internal cavity size (6 A°) [17] allows the drugs to fit well in the 2-HP-β-CD cavity and strongly bond to its donation sites.

In order to investigate the superior features offered by the SC-ISEs, two different configurations were prepared: a conventional liquid inner contact (sensors 1 and 2) and solid-contact glassy carbon ones (sensors 3 and 4) for the determination of CLO and PHE in binary mixtures, in the presence of their corresponding degradation products and in rabbit aqueous humor. A comparative study was performed regarding detection limits, concentration ranges, and the effect of the internal solution on the results.

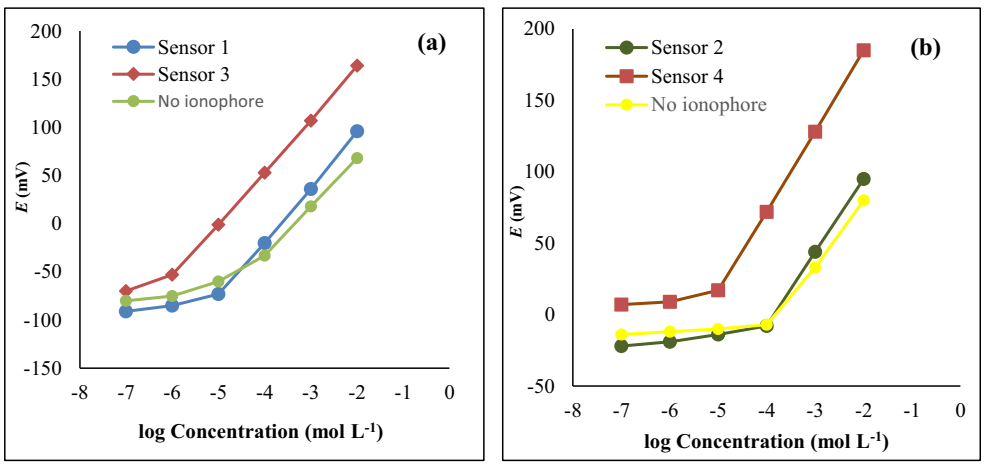

Fig. 2 Profile of the potential in mV versus log molar concentration of **a** CLO and **b** PHE using the proposed sensors

Sensor calibration and dynamic response time

The performance characteristics of the four proposed sensors at optimal pH and temperature were systematically examined according to IUPAC standards [14]. Table 1 shows the calculated metrological and validation parameters of the proposed sensors.

The potentiometric responses of sensors were evaluated in the concentration range from 1.0×10^{-7} to 1.0×10^{-2} mol L^{-1} respective drug solutions. The calibration plots for a monovalent cation are shown in Fig. 2, the slope was computed from the linear part of the calibration graph. The average slopes of the calibration plots are 56.50, 51.51, 54.20, and 56.31 mV/concentration decade for sensors 1, 2, 3, and 4, in order.

The sensors displayed nearly constant potential readings within ± 2 mV for day-to-day measurements and the calibration slopes did not show any significant change even after continuous use for a period of 45, 21, 60, and 35 days for sensors 1, 2, 3, and 4, respectively. The life spans of the SC-ISEs, in general, were longer than the corresponding liquid contact electrodes which indicated that the ion-exchanger did not easily exude into the bathing solution which maintain the sensitivity of the sensors. The detection limits (LOD) were calculated according to the IUPAC recommendations where the concentration of the primary ion at the point of intersection

Table 1 Electrochemical response characteristics and validation parameters of the four investigated CLO and PHE sensors

Parameter	Electrode A (liquid contact)		Electrode B (solid contact)	
	Sensor 1 (CLO)	Sensor 2 (PHE)	Sensor 3 (CLO)	Sensor 4 (PHE)
Slope ± SD (mV/decade)[a]	56.50 ± 0.26	51.51 ± 1.36	54.20 ± 0.49	56.31 ± 0.77
SE of slope	0.93	0.43	0.50	0.62
Intercept (mV)[a]	207.18	197.80	270.80	297.11
SE of intercept	3.56	1.29	2.14	2.35
Correlation coefficient (r)	0.9996	0.9999	0.9998	0.9998
Response time (s)	10	20	5	8
Working pH range	2.5–6.0	2.0–7.5	2.5–6.0	2.0–7.5
Concentration range (mol L^{-1})	1.0×10^{-5} to 1.0×10^{-2}	1.0×10^{-4} to 1.0×10^{-2}	1.0×10^{-6} to 1.0×10^{-2}	1.0×10^{-5} to 1.0×10^{-2}
Stability (days)	45	21	60	3
Accuracy				
Mean ± SD[b]	100.63 ± 0.17	100.17 ± 0.62	99.67 ± 1.44	100.36 ± 0.52
Precision (± %RSD)				
Repeatability[c]	1.50	1.47	0.93	0.77
Inter-day precision[d]	1.62	1.81	1.68	1.63
Specificity				
Mean ± SD	100.46 ± 0.53[e]	101.56 ± 3.11[f]	99.93 ± 1.02[e]	100.26 ± 1.29[f] 99.70 ± 0.71[g]
LOD (mol L^{-1})[h]	5.01×10^{-6}	6.31×10^{-5}	3.98×10^{-7}	3.16×10^{-6}
Robustness[i]	1.25	1.31	0.67	0.91

[a] Average of five determinations

[b] The accuracy ($n = 5$), average of five different concentrations

[c] Intra-day precision, average of three different concentrations of three replicate each ($n = 9$) repeated three times within the same day

[d] Inter-day precision ($n = 9$), average of three different concentrations of three replicate each ($n = 9$) repeated on three successive days of 5.0×10^{-5}, 3.0×10^{-5}, and 1.0×10^{-5} mol L^{-1} respective drug solutions for sensors 1 and 4; 5.0×10^{-6}, 3.0×10^{-6}, and 1.0×10^{-6} mol L^{-1} for sensor 3; and 5.0×10^{-4}, 3.0×10^{-4}, and 1.0×10^{-4} mol L^{-1} for sensor 2

[e] Average %recovery of CLO in laboratory-prepared mixtures containing different ratios of the co-formulated drug PHE and in the presence of up to 90% of their possible degradation products

[f] Average %recovery of PHE in laboratory-prepared mixtures in the presence of up to 40 and 60% of its oxidative degradation products for sensors 2 and 4, respectively

[g] Average %recovery of PHE in laboratory-prepared mixtures containing different ratios of CLO

[h] Limit of detection (according to the IUPAC definition, measured by interception of the extrapolated arms of non-responsive and the Nernstian segments of the calibration plot of Fig. 2)

[i] Robustness, %RSD of the previously determined concentrations of three replicates each ($n = 9$) under variations in method parameters (pH of the background buffer and temperature)

of the extrapolated lines of the Nernstian (high concentration) and non-responsive (low concentration) segments of the calibration curve could be considered as an "attainable" detection limit under the stated experimental conditions [14]. Table 1 shows the capability of sensors 3 and 4 to detect CLO and PHE in very dilute solutions down to 0.398 and 3.16 μmol L^{-1}, respectively, which is approximately one order of magnitude lower than the conventional liquid inner contact sensors (sensors 1 and 2). These findings agree with the idea that the elimination of the inner solution in SC-ISEs results in diminishing of ion flux from the membrane to the sample during measurements of dilute samples.

Response time is a critical parameter for analytical applications of ISEs that license accurate potentiometric measurements in a very short time. In this study, the practical response time was recorded after increasing drug concentration by up to tenfold. The time required for the sensors to reach steady *emf* values (± 0.1 mV) was found to be 10, 20, 5, and 8 s for sensors 1, 2, 3, and 4, respectively.

The characteristics of SC-ISEs outshine the conventional liquid inner contact electrodes due to the removal of the internal solution.

Effect of pH and temperature

The potential of the fabricated sensors over various pH ranges was examined. A pH value within a well-defined constant range of 2.5–6.0 for CLO and 2.0–7.5 for PHE was found to be optimum, from the perspective of sensor performance, chemical form of CLO and PHE in test solutions and stability of target ions. Figure 3a, b shows the potential-pH profiles for 1.0×10^{-3} and 1.0×10^{-4} mol L^{-1} CLO and PHE solutions using the described sensors for the studied drugs. The minor change in potential readings at pH values below the previously mentioned ranges is due to the interference of hydronium ions or sensor shocking, while the sharp drop in potential readings at higher pH values could be credited to the formation of non-protonated amino group of CLO and PHE and the possible degradation of the CLO molecules. Therefore, phosphate buffer pH 4.0 was used throughout the measurements of the investigated sensors at which both drugs are in fully ionized form.

Moreover, results obtained upon studying the effect of temperature revealed that the response of the developed sensors exhibited a minor rise in *emf* values with increasing temperature in the range of 25 to 35 °C. However, the calibration plots attained were parallel; also the LOD, slopes and response time did not fluctuate significantly with variations of temperature, demonstrating that the fabricated membranes are thermally stabile up to 35 °C.

Sensor selectivity

The effect of interfering substances on the performance of the proposed sensors was studied using a separate solution method [14]. Table 2 shows the potentiometric selectivity coefficients of the proposed sensors in the presence of possible degradates, co-formulated drugs, diluents, excipients, and also some other inorganic cations (K^+, Na^+, NH_4^+ and Ca^{2+}) that are usually found in biological fluids.

An explanation for the high selectivity of the developed ISEs towards CLO (sensors 1 and 3) could be attributed to its higher lipophilicity (log*P* 2.32) compared to other organic ions or co-formulated drug (phenylephrine hydrochloride, log*P* − 0.03). It is also important to highlight that the hydrolytic degradation products did not show any significant interference with the parent drug. The most probable reason is the lack of electroactive group (i.e., cationic moiety) [7] that results in decreasing partitioning to the organic membrane compared to CLO. This remarkably high discrimination facilitates the development of CLO-ISEs as stability-indicating method. Reviewing other analytical methods, this point is a challenging analytical task. UV-spectrophotometry was unable to afford insight into the degradation process due to the complete overlap of the absorption spectra of CLO and its alkali induced degradation products, as shown in Supporting

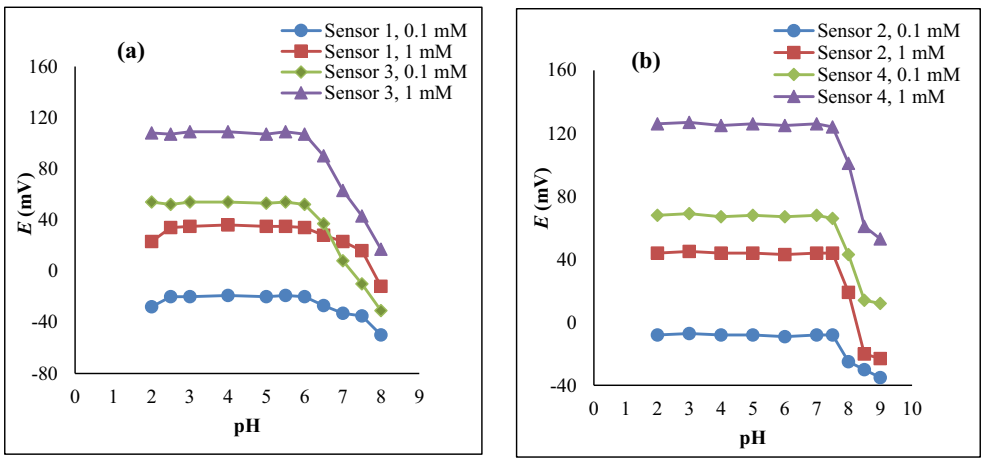

Fig. 3 Effect of pH on the response of the proposed sensors for **a** CLO and **b** PHE

information, Fig. S2. Furthermore, the reported chromatographic methods, although offer a high degree of specificity, suffer from either instrumentation limitation, prohibitive cost or technical difficulty, in addition to, the resulting contamination of the environment with organic solvents. The limitations associated with these techniques challenge their use in routine analysis.

Notably, PHE sensors (2 and 4) with the exception of CLO and its structurally related oxidative degradation products, the selectivity coefficients obtained for all other cations are in the order of 10^{-2} or smaller, indicating that they do not disturb the functioning of PHE-ISE significantly. This could be explained by the fact that these ions are easily exchanged into the organic membrane phase, in addition to the lower lipophilic character of PHE that affects its partition coefficient into the membrane. Remarkably, PHE SC-ISE (sensor 4) is at least 10–100 times more selective and displayed lower response for the potentially interfering species and co-formulated drug (CLO) than the conventional liquid contact one (sensor 2). The proposed membrane sensors seem to be reasonably selective towards phenylephrine hydrochloride, as shown in Table 2.

Also, the selectivity towards some common analytes and inorganic cations present in biological samples (Na^+, K^+, glucose and glycine) has been investigated. Really, the ISE preference displayed to CLO and PHE was credited to the relative hydrophilicity and limited ion exchange property of these ions into the lipophilic base.

Potentiometric determination of laboratory-prepared mixtures containing different ratios of CLO and PHE in the presence of their possible degradation products

The behavior and ability of the proposed sensors to distinguish the primary ion from the potentially interfering ions present, simultaneously at the same time was assessed.

Complete degradation of CLO was induced by standing with 0.1 mol L^{-1} NaOH for 2 h at room temperature (Supporting Information, Fig. S3), whereas an oxidative degraded phenylephrine hydrochloride sample was prepared by reflux with 3% H_2O_2, for 3 h. The reported oxidative degradation pathway of PHE is presented in Supporting information, Fig. S4. The resulting degradation products were isolated and subjected to MS scan for their identification [7].

Sensors 1 and 3 could be successfully used for selective quantification of CLO in the presence of co-formulated drug PHE and in the presence of up to 90% of their possible degradation products, in spite of their closely related structures as shown in Table 1. This is due to the presence of 2-HP-β-CD in the sensors structure that allows the formation of well-fitting inclusion cavity for the selected drug. Such results encourage the use of both sensors for stability studies and the assay of CLO in different pharmaceutical formulations and biological samples.

Upon analysis of synthetic mixtures containing different ratios of intact PHE and its oxidative degradation products, the results show that sensor 4 could be used for selective determination of intact PHE in the presence of up to 60% of its oxidative degradation products. Sensor 2 suffers from high interference when the degradation products concentration reaches about 40% (Table 1).

The main complications in the analysis of PHE in the presence of its co-formulated drug CLO are the similar cationic properties displayed by both drugs, with large difference in their lipophilic character in favor of CLO resulted in significant interference especially at high concentration levels of CLO. Analysis of synthetic mixtures containing different ratios of PHE and CLO showed that, although sensor 4 is far more sensitive to the interfering ion than to the primary ion, it could be successfully applied for selective determination of PHE only in the presence of lower quantities of CLO without prior separation (Table 1).

Detailed results obtained upon analysis of synthetic mixtures containing different ratios of CLO, PHE, and their possible degradation products in different ratios including the market ratios are summarized and presented in Supporting information, Tables S5–7.

Potentiometric determination of CLO and PHE in pharmaceutical formulations

The proposed sensors were employed for selective assaying of both CLO and PHE in their ophthalmic preparations. The susceptible preservatives and excipients normally used in eye formulations did not show any interference. Thus, analysis was carried out without prior treatment or extraction.

Table 3 showed that CLO sensors (sensors 1 and 3) were successfully employed for the selective determination of CLO either in its single or combined pharmaceutical formulation with PHE (although present at low concentration, 50 mg CLO versus 500 mg PHE per milliliter) without prior treatment or separation, as demonstrated by the accurate and precise percentage recoveries. This could be attributed to the higher lipophilicity of CLO in comparing with PHE that affects its hydration enthalpy and partition coefficient into the membrane.

No membrane electrode has been suggested for determination of PHE till now, in the presence of its co-formulated drug, CLO. A net result that the proposed PHE liquid inner contact sensor (sensor 2) suffered highly interference from CLO, was also experienced upon analysis of Cyclophrine® eye drops as shown in Table 3. An approach to fine-tune the selectivity of the proposed PHE-ISEs based on the effect pH adjustment was carried out. However, this line did not help much. Finally, PHE SC-ISE sensor (sensor 4) was proved to be the one of choice for this determination, keeping CLO in micro molar concentrations, directly without prior separation, Table 3.

Table 2 Potentiometric selectivity coefficient ($K^{pot.}_{primary\ ion,\ interferent}$) of the four investigated sensors using separate solution method (SSM)

Interferents[b]	$K^{pot.}_{primary\ ion,\ interferent}$[a]			
	Electrode A (liquid contact)		Electrode B (solid contact)	
	Sensor 1 (CLO)	Sensor 2 (PHE)	Sensor 3 (CLO)	Sensor 4 (PHE)
K^+	8.15×10^{-3}	13.40×10^{-2}	1.44×10^{-3}	3.38×10^{-2}
Na^+	8.85×10^{-3}	1.96×10^{-2}	5.20×10^{-4}	6.88×10^{-3}
NH_4^+	4.61×10^{-3}	6.54×10^{-2}	6.16×10^{-4}	4.96×10^{-2}
Ba^{2+}	9.60×10^{-3}	2.80×10^{-2}	1.33×10^{-4}	1.18×10^{-2}
Ca^{2+}	7.83×10^{-3}	3.06×10^{-2}	3.12×10^{-4}	7.47×10^{-3}
Mg^{2+}	6.13×10^{-3}	8.75×10^{-3}	6.71×10^{-4}	3.15×10^{-3}
Sr^{2+}	7.52×10^{-3}	2.14×10^{-2}	7.62×10^{-4}	9.97×10^{-3}
Lactose	3.19×10^{-3}	1.31×10^{-2}	9.83×10^{-4}	9.18×10^{-3}
Glucose	2.40×10^{-3}	1.14×10^{-2}	5.66×10^{-4}	8.45×10^{-3}
Glycine	3.92×10^{-3}	2.24×10^{-2}	4.98×10^{-4}	5.60×10^{-3}
Urea	1.11×10^{-3}	3.06×10^{-2}	3.55×10^{-4}	7.79×10^{-3}
CLO[c]	–	1.22×10^{3}	–	4.07×10^{1}
PHE[c]	5.89×10^{-3}	–	4.38×10^{-4}	–
CLO alkali degradates	5.43×10^{-3}	0.57×10^{-2}	1.57×10^{-3}	1.70×10^{-2}
PHE oxidative degradates[c]	1.16×10^{-2}	1.40×10^{1}	2.20×10^{-3}	1.78×10^{-1}

[a] Each value is the average of three determinations

[b] All interferents are in the form of 1.0×10^{-3} mol L^{-1} aqueous solution

[c] Calibration curves were obtained by successive dilution and near-Nernstian response was confirmed in the concentration range where selectivity was measured

Potentiometric determination of CLO and PHE in rabbit aqueous humor

CLO and PHE were successfully determined in spiked rabbit aqueous humor without prior treatment or cleanup step by the proposed sensors (Table 3). The results show that the proposed SC-ISEs (sensors 3 and 4), based on their superior selectivity and lower LOD, are more reliable and give stable results with very good accuracy and high-percentage recoveries for the simultaneous determination of CLO and PHE at the market product ratio in spiked rabbit aqueous humor without preliminary separation procedures as shown in Table 3. The

Table 3 Determination of CLO and PHE in pharmaceutical formulations and spiked rabbit aqueous humor by the suggested potentiometric procedure

Application		Found % ± SD[a]			
		Electrode A (liquid contact)		Electrode B (solid contact)	
		Sensor 1 (CLO)	Sensor 2 (PHE)	Sensor 3 (CLO)	Sensor 4 (PHE)
Pharmaceutical formulations	Colircusi Ciclоplejico® eye drops (batch no. 5LCG2A) (each mL was labeled to contain 10 mg CLO)	99.86 ± 0.71[b]	–	100.41 ± 1.19[b]	–
	Cyclophrine® eye drops (batch no. 1560308) (each 5 mL was labeled to contain 50 mg CLO and 500 mg PHE)	99.51 ± 0.38[c]	129.35 ± 0.74[d]	99.15 ± 0.94[d]	99.94 ± 1.33[d]
Biological samples	Rabbit aqueous humor	100.58 ± 0.94[c]	107.38 ± 0.57[e]	101.09 ± 1.46[d]	101.04 ± 1.96[d]

[a] Average of five determinations

[b] Calculated as 1.0×10^{-4} mol L^{-1} CLO

[c] Calculated as 1.0×10^{-4} mol L^{-1} CLO and 1.63×10^{-3} mol L^{-1} PHE

[d] Calculated as 6.13×10^{-6} mol L^{-1} CLO and 1.0×10^{-4} mol L^{-1} PHE

[e] Calculated as 1.0×10^{-4} mol L^{-1} PHE

Table 4 Statistical comparison of the results obtained by the proposed method and those obtained by the official ones for the determination of cyclopentolate hydrochloride and phenylephrine hydrochloride in pure powder forms

Value	Proposed ISE method				Official method [2]	
	Electrode A (liquid contact)		Electrode A (solid contact)			
	Sensor 1 (CLO)	Sensor 2 (PHE)	Sensor 3 (CLO)	Sensor 4 (PHE)	CLO[(a)]	PHE[(b)]
Mean	99.92	100.01	99.92	99.97	100.10	99.81
SD[(c)]	1.09	0.48	0.89	0.54	1.32	1.07
% RSD[(c)]	1.09	0.48	0.89	0.54	1.32	1.07
Variance	1.19	0.23	0.79	0.29	1.74	1.14
Student's *t* test (2.306)	0.235	0.378	0.187	0.297	–	–
F value (6.39)	1.46	4.96	2.20	3.93	–	–

The values in the parenthesis are the corresponding theoretical values of *t* and *F* at $P = 0.05$

[(a)] Official method is a potentiometric titration method by dissolving 0.250 g CLO in a mixture of 1.0 mL 0.1 M hydrochloric acid and 50.0 mL ethanol (96%). Carry out a potentiometric titration, using 0.1 M sodium hydroxide

[(b)] Official method is a potentiometric titration method by dissolving 0.150 g PHE in a mixture of 0.1 mL 0.1 M hydrochloric acid and 80.0 mL ethanol (96%). Carry out a potentiometric titration, using 0.1 M ethanolic sodium hydroxide

[(c)] Standard deviation and percentage relative standard deviation of five determinations for the proposed sensors and the official methods

response time of all the proposed sensors is instant (within 20 s). Sensors were rapidly transferred back and forth between the aqueous humor samples and the deionized water to save the sensing component from any matrix components that may adhere to its surface. It is concluded that the proposed sensors could be successfully applied to in vitro studies and for clinical use.

To examine the accuracy of the proposed sensors, the obtained results were also compared to those obtained by applying the official method [2] for pure CLO and PHE determinations (Table 4). Statistical analysis of the results between the proposed and BP official methods using Student's *t* test and *F* ratio revealed no significant difference between them regarding accuracy and precision. These official titrimetric methods involve the use of hazardous chemicals and solvents using a mixture of 0.1 mol L^{-1} hydrochloric acid and alcohol as a solvent with potentiometric detection of end point versus 0.1 mol L^{-1} NaOH. Noticeably, the proposed potentiometric ISE method is straight forward, green environmentally friendly method that cut short time and effort, and thus reducing cost per sample.

Conclusion

In general, the use of ISEs for drug-related analytical investigations is a promising concept for measurements that aimed at decreasing the struggle of analysis in terms of time, cost, expertise, portability, and organic waste generated with separation methodologies such as HPLC. Moreover, usage of the proposed sensors compromises a great advantage of eliminating any need for drug pretreatment or separation steps. Therefore, it could be used for routine analysis of CLO and PHE in quality-control laboratories.

A comparative potentiometric study between two kinds of electrodes (conventional, liquid inner contact, and a glassy carbon solid-contact one), constructed for novel simultaneous determination of CLO and PHE, was conducted. Clearly, the glassy carbon solid-contact sensors showed advantageous performance characteristics and superior features relative to their corresponding liquid contact ISEs, in terms of shorter response time, longer operative lifetimes, enhanced selectivity, and lower detection limits. The proposed sensors are applicable as stability indicating assay methods for in-line pharmaceutical industries and biological studies for accurate monitoring of the drugs without interference from dosage form excipients or aqueous humor ions or proteins.

Compliance with ethical standards

Ethical approval This work was approved by the Institutional Research Ethical Committee at Faculty of Pharmacy, Cairo University.

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