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Green Ion Selective Electrode Potentiometric Application for the Determination of Cinchocaine Hydrochloride in Presence of Its Degradation Products and Betamethasone Valerate: A Comparative Study of Liquid and Solid Inner Contact Ion-Selective Electrode Membranes

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The sustainable and green chemistry principles enable scientists to protect and benefit the economy, people and the planet by finding creative and innovative ways to reduce waste, conserve energy, and discover replacements for hazardous substances. In this work, an environmentally friendly ion selective electrode (ISE) potentiometric method was developed for the determination of cinchocaine hydrochloride (CIN) in presence of its degradation products either in bulk powder or in its combined pharmaceutical formulation with betametahsone valerate. Two novel CIN-selective electrodes were fabricated and evaluated. The fabrication of electrodes was based on sodium tetrakis [3,5-bis (trifluoromethyl)phenyl]borate as a cationic exchanger in a PVC matrix with 2-nitrophenyl octyl ether (2-NPOE) as a plasticizer and using 2-hydroxy propyl-β-cyclodextrin (2-HP β-CD) as an ionophore. A comparative study was conducted using two designed CIN-selective electrodes; a conventional liquid inner contact, sensor 1, and a glassy carbon solid contact electrode, sensor 2. Sensor 1 has a linear dynamic range of 3.0×10^{-5} mol L^{-1} to 1.0×10^{-2} mol L^{-1}, with a Nernstian slope of 54.89 mV/decade and a detection limit of 5.01×10^{-6} mol/L. Sensor 2 shows linearity over the concentration range of 1.0×10^{-5} mol L^{-1} to 1.0×10^{-2} mol L^{-1}, with a Nernstian slope of 57.01 mV/decade and a limit of detection of 2.51×10^{-6} mol L^{-1} which is much improved as a result of diminishing ion fluxes in this solid contact ion-selective electrode. The present electrodes show clear selectivity for CIN from several inorganic, structurally related organic molecules, sugars, co-formulated drug, some common drug excipients and its degradation products. The results obtained by the proposed sensors were statistically analyzed and compared with those obtained by official method. No significant difference for either accuracy or precision was observed.
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Pharmaceutical product quality is of eminent importance for patient safety. The presence of impurities may alter the efficacy and safety of pharmaceuticals. Impurities and potential degradation products can cause changes in the chemical, pharmacological and toxicological properties of drugs and thereby have a significant impact on product quality and safety. Cinchocaine hydrochloride (CIN), a local anesthetic agent, is chemically; 2-butoxy-*N*-[2-(diethylamino)ethyl]quinoline-4-carboxamide hydrochloride,[1] Figure 1a. It is sometimes co-formulated with the glucocorticoid anti-inflammatory drug; betamethasone valerate (BMV) which is chemically known as 9-fluoro-11β, 21-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl pentanoate,[1] Figure 1b. The combination of CIN and BMV, formulated as ointment, is used for local treatment of hemorrhoids. CIN is known to be labile for acid degradation, while it shows relative stability toward alkali, oxidative, photolytic and thermal stress conditions.[2–5] Few ion selective electrodes have been reported for the determination of CIN either singly or in combined dosage forms.[6–8] These sensors did not investigate the selective determination of intact CIN in presence of its labile degradation products or betamethasone as a co-formulated drug.

In addition of being an environmentally friendly technique, potentiometric measurements with ISEs are considered as a promising analytical tool for their attractable characteristics such as simple design, ease of construction, portability, low energy consumption, reasonable selectivity, fast response time, limited sample pretreatment, rapidity, being non-destructive and adaptability to small sample volumes. Design of sensors with refined characteristics of a certain chemical species is quite challenging in chemical research. To this end, we are undertaking this work that involves the design, study and comparison of new sensors to determine CIN. The construction of a polymeric membrane, ion-selective electrode traditionally required a relatively high concentration of the ion of interest in the inner filling solution; however, experimental evidence suggested that this has a deteriorating influence on the detection limit.[9] Mathison and Bakker[10] showed that an increased concentration of the primary ion in the inner solution leads to its extraction from there together with its counter ions forming ion fluxes from the membrane to the sample. This process changes the ion activity at the phase boundary thus significantly worsening the detection limit. One strategy to counteract this behavior is elimination of the inner solution by using a solid inner contact. In solid-contact SC-ISEs, the sensing membrane is sandwiched between the sample solution and an SC- ISEs.

To enlighten this concept, we developed two novel sensors namely; a conventional liquid contact (sensor 1) and a solid contact glassy carbon one (sensor 2). In addition, a comparative study was conducted concerning detection limits, concentration ranges, sensors stability

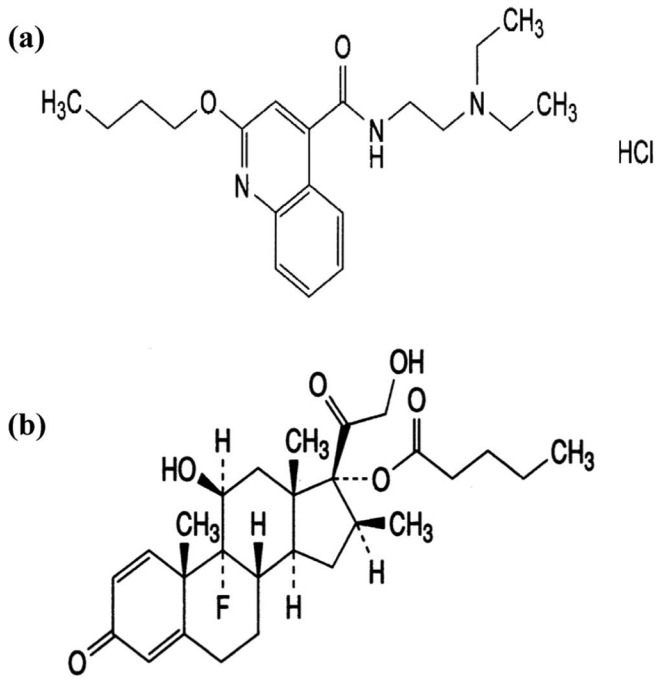

Figure 1. Chemical structures of cinchocaine hydrochloride (a) and betamethasone valerate (b).

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and the effect of the internal solution. These sensors were used for the selective determination of CIN in presence of its degradation products either in bulk powder or pharmaceutical formulations.

Experimental

Instruments.—Potentiometric measurements were carried out using an Ag/AgCl double-junction-type external reference electrode (Thermo Scientific Orion 900200, MA, USA; 3.0 M KCl saturated with AgCl as an inner filling solution and 10% KNO_3 as a bridge electrolyte) and Jenway digital ion analyser (model 3505; Essex, UK). A Jenway pH glass electrode (Essex, UK) was used for pH adjustments. Magnetic stirrer, Bandelin Sonorox, Rx510S (Budapest, Hungary). Glassy carbon electrode was used as the solid contact (3 mm diameter, CH Instruments, Texas, USA).

A Soniclean 160T sonicator (Soniclean, Thebarton, Australia) was used for extraction of drugs from pharmaceutical formulations.

Materials and Reagents

Pure standards.—Cinchocaine hydrochloride standard was kindly supplied by Alexandria Co. Pharmaceuticals, Alexandria, Egypt. Its purity was assessed and found to be 100.05% ± 1.32 according to BP official method.[1] Betamethasone valerate standard was kindly supplied by GlaxoSmithkline S.A.E., Cairo, Egypt. Its purity was checked and found to be 100.46% ± 1.17 according to BP official method.[1]

Pharmaceutical formulations.—Supraproct-S ointment (Batch No. 0014) was manufactured by Julphar, Gulf Pharmaceutical Industries, Ras Al Khaimah, UAE. Each gram was labeled to contain 5 mg of CIN and 1 mg of BMV.

Chemicals.—All chemicals and reagents used throughout this work were of analytical grade, and water used was bi-distilled.

Polyvinyl chloride (PVC) and 2-nitrophenyl octyl ether (NPOE) were obtained from Fluka Chemie GmbH (Steinheim, Germany). Sodium tetrakis [3,5-bis(trifluoromethyl)phenyl]borate (NaBARF) was obtained from Alpha Aesar GmbH & Co KG (Karisruhe, Germany). 2- hydroxypropyl β-cyclodextrin (2-HP β-CD), tetrahydrofuran (THF), monobasic sodium phosphate, sodium hydroxide, hydrochloric acid, chloroform, acetonitrile and n-hexane were purchased from Sigma-Aldrich (Steinheim, Germany), potassium chloride Fluka AG, Buchs SG (Switzerland). Glacial acetic acid, toluene, ethanol (El Nasr Pharmaceutical Chemical Co., Egypt) were used.

Degraded Samples

Cinchocaine hydrochloride acid induced degradation products preparation.—Into a 100-mL rounded flask, a mass of 20 mg of CIN was transferred; 20 mL of 2 M hydrochloric acid were then added and refluxed for 2 hrs. After reflux, the solution was cooled. The pH was then adjusted to 7 with a pre-calculated amount of 2 M sodium hydroxide. Complete degradation was confirmed by HPTLC.[5]

Standard solutions.—CIN stock standard solution (1.0×10^{-2} mol L^{-1}): It was prepared by accurately transferring 0.380 g CIN into a 100-mL volumetric flask, which was dissolved in a sufficient amount of phosphate buffer, pH 4.0 and then the volume was completed to mark with the same solvent.

CIN working standard solutions: Different solutions of varying strengths (1.0×10^{-7} - 1.0×10^{-3} mol L^{-1}) were freshly prepared by serial dilutions from the stock solution using a phosphate buffer at pH = 4.

Procedures

Fabrication of membrane sensors.—Preparation of conventional inner contact electrodes, sensor 1: in a glass petri dish (5-cm diameter),

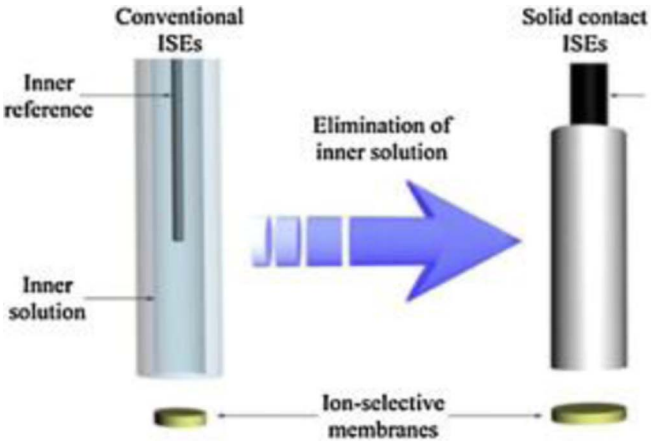

Figure 2. Comparison between conventional ion-selective electrodes and solid contacts ion-selective electrodes.

10 mg of NaBARF was thoroughly mixed with 0.35 mL of o-NPOE, 190 mg PVC and 10 mg 2-HP β-CD. The membrane components were dissolved in 6 mL THF by stirring using a glass rod. The petri dish was then covered with filter paper and left to stand overnight at room temperature to allow solvent evaporation, leaving a homogeneous, flexible and transparent membrane. A master membrane of about 0.1 mm in thickness was obtained. From the prepared membrane, a disk (about 8 mm in diameter) was cut using a cork borer and was then fixed using THF to a transposable PVC tip that was clipped into the end of an electrode glass part. The electrode was then filled with an internal solution of equal volumes of 1.0×10^{-2} mol L^{-1} CIN and 1.0×10^{-2} mol L^{-1} KCl. Ag/AgCl wire (1 mm diameter) was used as an internal reference electrode. The sensor was conditioned by soaking in 1.0×10^{-2} mol L^{-1} CIN stock standard solution for 24 hours, and it was stored in the same solution when not in use.

Fabrication of SC-ISE electrodes, sensor 2: twenty μL of the previously prepared liquid ion-selective membrane solution in THF were directly applied using a micropipette on a glassy carbon electrode that has been previously mechanically polished and cleaned with water. The entire outer membrane was then allowed to evaporate overnight before the solid contact electrode was conditioned in 1.0×10^{-2} mol L^{-1} CIN. Schematic diagrams of (a) liquid and (b) solid inner contact ion-selective electrode membranes are illustrated in Figure 2.

Sensors calibration.—The conditioned sensors were calibrated by separately transferring 20-mL aliquots of drug solutions (1.0×10^{-7} to 1.0×10^{-2} mol L^{-1}) prepared in phosphate buffer solution pH 4.0 into a series of 50-mL beakers. The membrane sensors, in conjunction with the Ag/AgCl reference electrode, were immersed in the above test solutions and allowed to equilibrate while stirring. The potential difference (emf) between the membrane sensor (indicator electrode) and the reference electrode was recorded after stabilizing to ±1 mV, and the electromotive force was plotted as a function of the logarithm of CIN concentrations in buffer solution of pH 4.0 at 25°C.

Effect of pH and temperature on electrode response.—The effect of pH on the response of the investigated electrodes (potential) was studied using 1×10^{-3} and 1×10^{-4} mol L^{-1} aqueous CIN standard solutions at different pH values, ranging from 2.0 to 7.5. The obtained potential at each pH value was recorded.

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

Sensors selectivity.—The potential response of the two proposed sensors in the presence of a number of related substances was studied and the potentiometric selectivity coefficient, the ability of the sensing membrane in discriminating the primary ion against other ion of the

same charge sign, ($K^{pot}_{CIN,\ interferent}$) was calculated to estimate the degree to which a foreign substance would interfere with the response of the electrodes to their primary ion (CIN with sensors 1 and 2). The selectivity coefficients were evaluated according to International Union of Pure and Applied Chemistry (IUPAC) guidelines using the separate solutions method (SSM)[11] using the following equation:

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

where $K^{pot}_{A,B}$ is the potentiometric selectivity coefficient, E_1 is the potential measured (mV) in 1.0×10^{-3} mol L^{-1} of CIN 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).

Direct potentiometric determination of CIN in pharmaceutical dosage forms.—Into a 100-mL beaker, an amount of 2.0 g Supraproct–S ointment was accurately weighed and dispersed into 20 mL n-hexane with aid of stirring and sonication for 15 minutes. The solution was quantitatively transferred into a 150-mL separating funnel. Shaking was done for 3 minutes till complete dispersion of the ointment. The solution was extracted three times, each with 5 mL of the phosphate buffer, pH 4.0. The lower layer, containing the active ingredients, was then transferred, quantitatively, into a 25-mL volumetric flask. The volume was completed to mark with the same solvent. The concentration of this solution is claimed to be 1.05×10^{-3} mol L^{-1} CIN. The potentiometric measurements were performed using the proposed sensors in conjunction with the Ag/AgCl reference electrode, and the potential readings were compared to the calibration plots.

Analysis of synthetic mixtures.—Potentiometric measurements were carried out in different synthetic mixtures containing a fixed concentration of CIN (1.0×10^{-4} mol L^{-1}) while varying concentrations of either its acid degradation products or co-formulated drug (betamethasone valerate) in phosphate buffer (pH 4.0). The potential of each mixture was recorded with the two sensors and the concentration of CIN was calculated from the corresponding regression equations.

Results and Discussion

Concerning the stability of CIN, the intact drug was subjected to acid and alkali hydrolysis, UV light, thermal and oxidation. Degradation processes were confirmed by HPTLC.[5] It was found that CIN is susceptible to degradation under acidic conditions giving two degradation products while it shows relative stability toward alkali, photolytic, oxidative and thermal stress conditions. Figure 3 shows the reported acid degradation of the drug. The proposed degradation pathway was suggested to set forth through the formation of N-[2-(diethylamino)ethyl]-2-hydroxyquinoline-4-carboxamide (Deg. I). Further degradation of (Deg. I) gives 2-hydroxyquinoline-4-carboxylic acid (Deg. II).[2,4–5]

Design and development of new electrodes to measure various chemical species, such as CIN, is a prospering area of research. It is valuable to produce new fabricated electrodes with competitive properties. With these points in mind, we have extensively worked in the design and characterization of these new sensors: a conventional, liquid inner, contact sensor and a solid contact, glassy carbon sensor of CIN and then compared their properties in light of these considerations.

Performance characteristics of the proposed ionophore-based sensors.—It is well known that the performance characteristics of ISEs based on ion-exchangers depend, to a large extent, on the nature of these ion-exchangers and their lipophilicities,[12] the type of solvent mediator[13] and any additives used.[14] The fact that CIN behaves as a cation suggests the use of an ISE membranes which exhibit cation exchange capacity. A lipophilic cationic exchanger; Sodium tetrakis [3,5-bis(trifluoromethyl)phenyl]borate (NaBARF), was used for fabrication of the proposed sensors. Lipophilic mobile ion-exchanger

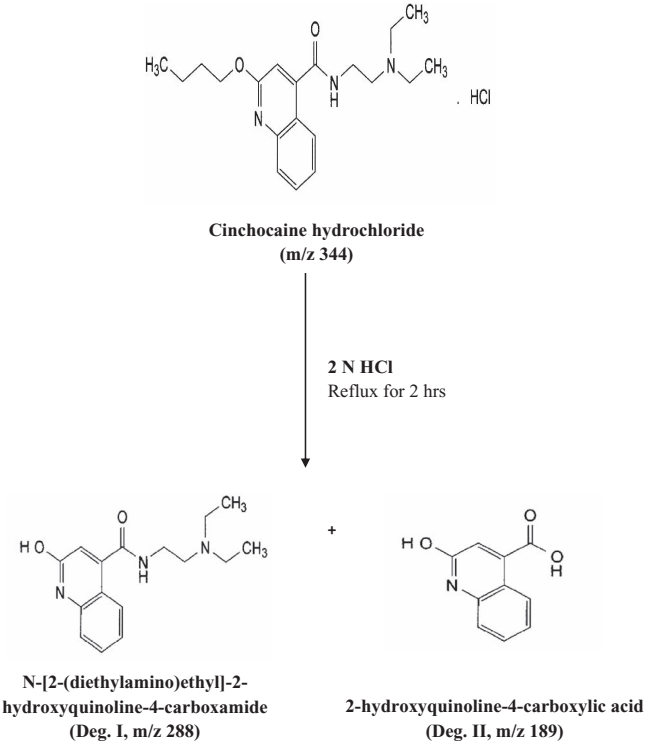

Figure 3. The suggested pathway for cinchocaine hydrochloride acid induced degradation.

sites play a key role as added components of the ISE membranes. Their main function is to render the ion selective membrane permselective in order to observe Nernstian response slopes. The membrane sensors were initially conditioned in 1×10^{-2} mol L^{-1} CIN for 1 day to replace the original exchangeable counter ion (Na^+) of the ion exchanger with CIN, so the membrane potential becomes responsive to the activity of this analyte in the sample phase.

The solvent mediator, in particular, has a dual function: it acts as a liquefying agent, making the membrane material workable, thus enabling homogenous solubilization and modification of the distribution constant of the ion-exchanger used and sustaining these characteristics on continued use.[15] For a plasticizer to be adequate for use in sensors, it should possess certain properties and characteristics, such as having high lipophilicity, high molecular weight, low tendency for exudation from the membrane matrix, low vapor pressure and high capacity to dissolve the substrate and other additives present in the membrane.[16] The values of dielectric constants, lipophilicity and molecular weight of the aromatic plasticizer NPOE (ε_r 24, P_{TLC} 10.2, m.w. 435)[17] favors its use to plasticize the membrane and adjusts both the membrane permittivity and the mobility of the ion-exchanger sites to facilitate the inclusion of organic molecules by competitive inclusion and give the optimal selectivity and sensitivity.

Efforts to improve ISEs characteristics have been proposed through the use of species capable of molecular recognition (ionophores). Inclusion complexation and complementary ionic or hydrogen bonding are the two main strategies for the synthesis of the host molecules. Different macrocyclic ionophores have been proposed for use with organic drug guest molecules, such as crown ethers, calixarenes, cyclodextrins and porphyrins.[18,19] In the present work, the ionophore 2-hydroxy propyl β-cyclodextrin was selected based on its outstanding complexation properties. The hydrophobicity of the cyclodextrin cavity provides a microenvironment to an appropriately sized non polar part of the drug molecule to enter the cavity and form an inclusion complex. The host-guest complex is stabilized via intermolecular hydrogen bonding, hydrophobic interactions, and van der Waals forces.[20]

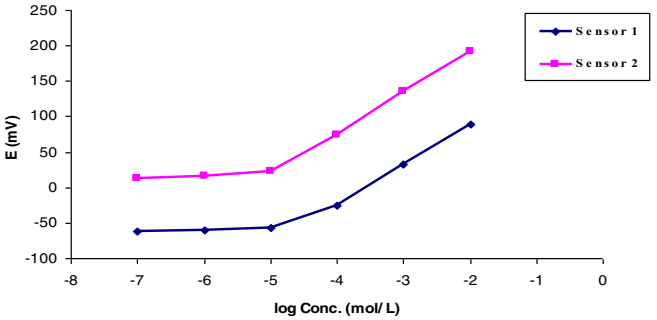

Figure 4. Profile of the potential in mV versus log concentrations of CIN in mol L^{-1} obtained with sensors 1 and 2.

In order to investigate the superior features offered by the solid contact-ion selective electrodes. We have developed two different configurations; a conventional liquid inner contact (sensor 1) and a solid contact glassy carbon (sensor 2). A comparative study was conducted concerning detection limits, concentration ranges and the effect of the internal solution on the results. Figure 4 shows the calibration plots for both sensors. Compared to sensor 1, sensor 2 shows improved Nernstian response slope along with enhanced selectivity coefficients values and sensitivity.

Table I shows the electrochemical performance characteristics of the two proposed sensors (slope, working pH range, quantitation limit, detection limit, linear dynamic range, stability and response time) systematically evaluated with respect to the International Union of Pure and Applied Chemistry (IUPAC) recommendations.[11] Clearly, reproducible results were revealed by two different assemblies for each sensor 1 and 2 over a period of 30 and 45 days, respectively and the measurements were carried out at 25°C.

The electrode reproducibility in terms of slope and standard potential (E^0) was studied by carrying out a frequent calibrations by the same membrane intra-daily (n = 3) and inter-daily over a couple of weeks. The results obtained for sensor 1 and 2 regarding the slopes were 54.89 ± 0.72 and 57.01 ± 1.02 mV/decade, while for E^0 were 196.8 ± 2.5 and 305.27 ± 4.17 mV, respectively. The obtained results indicated that the constructed ISEs exhibited acceptable repeatability; however the slight change in the reproducibility among days makes it appropriate to carry out calibrations on daily-basis.

Detection limit was calculated for the two sensors according to the IUPAC recommendations[11] where the concentration of the primary ion at the point of intersection of the extrapolated lines of the Nernstian (high concentration) and non responsive (low concentration) segments of the calibration curve can be considered as an "attainable" detection limit under the stated experimental conditions. It was found that Sensor 2 can detect CIN in very dilute solutions down to 2.51×10^{-6} mol L^{-1}. These results revealed the superior characteristics of solid contact electrodes with respect to the conventional liquid electrode due to elimination of the internal solution.

The reported conventional liquid contact electrodes, where the ion selective membrane is sandwiched between sample solution and inner filling solution, have some inherent limitations such as mechanical complexity (i.e. high risk of solution leakage and the need for maintenance), limited to taking vertical measurements and difficult to miniaturize. In these regards, solid contact ion selective electrodes (SC-ISEs) should offer simple, rigid and robust sensors as well as miniaturization capability, long-term storage, transport, sterilization are also much easier with these sensors. Moreover, these sensors can operate in any position or configuration and their ability to integrate with microfabrication processes allowing for the mass fabrication of cost-effective sensors.[21]

Methods validation has been performed according to ICH guidelines.[22] The linearity of the proposed methods is evaluated by analyzing different concentrations of standard solutions of CIN in triplicates. The values of correlation coefficients are close to unity indicating good linearity.

The accuracy of the results is checked by applying the proposed sensors for determination of five different concentrations for each of pure CIN. The concentrations are obtained from the corresponding regression equations then the percentage recoveries are calculated (Table II).

The precision of the results is checked by applying the proposed methods for determination of three concentrations of pure CIN within their linearity ranges; where the concentrations are analyzed three times, intra-daily (repeatability), and analysis of three concentrations on three successive days to evaluate the intermediate precision. The concentrations are obtained from the corresponding regression equations then %RSD are calculated (Table II). Table II presents the validation parameters of the two proposed sensors.

Effect of pH, temperature and dynamic response time.—For reliable quantitative measurements with the ISEs, a careful investigation of the experimental conditions was performed. The potential stability of the proposed sensors over various pH ranges was examined. Practically, the results revealed a fairly stable potential over pH range 2.5-6 for both sensors (Fig. 5) taking in concern the target ion chemical form and the performance of sensor. At pH values below the stated range, a minor change in emf readings is likely credited to degradation of CIN in highly acidic media, while at higher values of pH, a consistent decrease in the emf values was observed, which is explained by the formation of non-protonated CIN in alkali media (un-ionized) and the OH^- ions penetrate the membrane. It is worth noting that above pH 7.5, the solution shows turbidity due to precipitation of free CIN base.

Additionally, the results obtained upon investigation of the temperature effect suggest that the electrodes exhibit a slight increase in potential with increasing temperature in the range of 25–35°C. However, the calibration plots obtained at different temperatures are parallel, and the limits of detection, slopes and response times do not vary significantly with temperature, indicating a reasonable thermal stability of the PVC membranes up to 35°C.

Dynamic response time is an important factor for analytical applications of ion-selective electrodes. In this study, practical response time was recorded by increasing CIN concentration up to 10-fold. The required time for the sensors to reach values within 1 mV of the final equilibrium potential was 20 and 5 s for sensors 1 and 2, respectively.

Selectivity of the proposed sensors.—The selectivity coefficient is a summary of information concerning interferences on the electrode response in analytical applications and depends on the selectivity of the ion-exchange process at the sensor-test solution interface and the mobility of the respective ions in the matrix of the sensor.

Table I. Electrochemical response characteristics of the two investigated CIN-selective sensors.

Parameter	Sensor 1 (liquid contact)	Sensor 2 (solid contact)
Slope (mV/decade)[a]	54.89	57.01
Intercept (mV)	196.8	305.27
LOD (mol L^{-1})[b]	5.01×10^{-6}	2.51×10^{-6}
Response time (s)	20 s	5 s
Working pH range	2.5- 6	2.5- 6
Concentration range (mol L^{-1})	3×10^{-5} to 1×10^{-2}	1×10^{-5} to 1×10^{-2}
Stability (days)	30	45
Correlation coefficient (r)	0.9995	0.9995

[a]Average of five determinations.
[b]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 Figure 4).

Table II. Assay validation parameters of the proposed sensors.

Parameter	Sensor 1 (liquid contact)	Sensor 2 (solid contact)
Linearity		
Slope[a]	54.89	57.01
SE of Slope	1.02	1.04
Intercept[a]	196.86	305.27
SE of Intercept	3.55	3.75
Correlation coefficient (r)	0.9995	0.9995
Range (mole/L)	3.0×10^{-5} to 1.0×10^{-2}	1.0×10^{-5} to 1.0×10^{-2}
Accuracy (Mean ± SD)[b]	100.05 ± 0.62	101.07 ± 0.51
Precision (%RSD)		
Repeatability[c]	1.08	1.24
Intermediate Precision[d]	1.40	1.44
Specificity and Selectivity		
Mean ± SD[e]	99.98 ± 1.33	100.08 ± 0.91
Mean ± SD[f]	100.74 ± 0.24	99.79 ± 0.26
Sensitivity		
LOD (mol L^{-1})	5.01×10^{-6}	2.51×10^{-6}
LOQ (mol L^{-1})	3.0×10^{-5}	1×10^{-5}
Robustness[g]	0.7	0.68

[a]Average of five determinations.
[b]The accuracy (n = 5), average of five different concentrations.
[c]The intra-day, % RSD of concentrations (1.0×10^{-2}, 1.0×10^{-3} and 1.0×10^{-4} mol L^{-1}) of 3 replicate each (n = 9) repeated three times within the day.
[d]The inter-day, % RSD of concentrations (1.0×10^{-2}, 1.0×10^{-3} and 1.0×10^{-4} mol L^{-1}) of 3 replicate each (n = 9) repeated on 3 successive days.
[e]Recovery of CIN in laboratory prepared mixtures containing its degradation products.
[f]Recovery of CIN in laboratory prepared mixtures containing betamethasone valerate.
[g]Robustness, RSD% of the previously determined concentrations of 3 replicate each (n = 9) under variations in method parameters (pH of the background buffer).

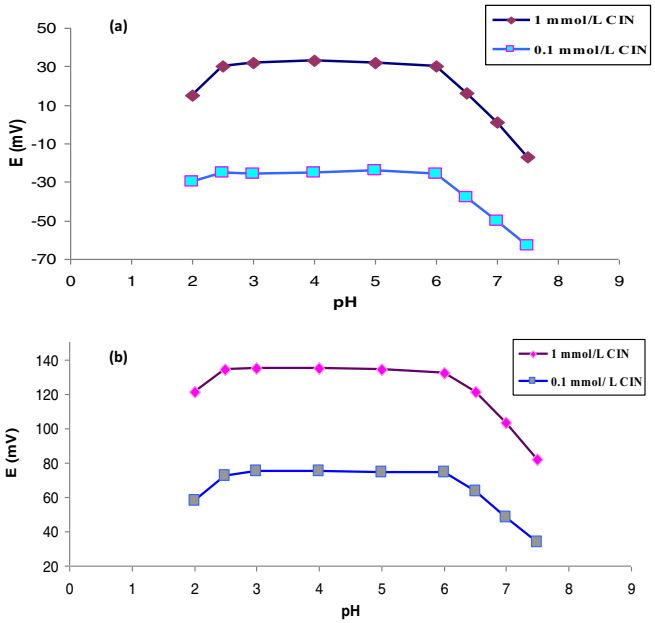

Figure 5. Effect of pH on the response of the proposed (a) liquid contact sensor 1 and (b) solid contact sensor 2.

Table III. Potentiometric selectivity coefficient ($K^{pot.}_{primary\ ion,interferent}$) of the proposed sensors by separate solution method (SSM).

	$K^{pot.}_{primary\ ion,\ interferent}$[a]	
Interferents[b]	Sensor 1 (liquid contact)	Sensor 2 (solid contact)
K^+	9.11×10^{-3}	6.96×10^{-4}
Na^+	3.06×10^{-3}	1.69×10^{-3}
NH_4^+	1.78×10^{-2}	7.24×10^{-3}
Ba^{2+}	1.71×10^{-2}	9.61×10^{-3}
Ca^{2+}	6.79×10^{-3}	4.12×10^{-3}
Mg^{2+}	2.38×10^{-3}	3.80×10^{-3}
Sr^{2+}	5.99×10^{-3}	4.84×10^{-3}
Lactose	1.32×10^{-2}	1.63×10^{-3}
Glucose	8.38×10^{-3}	2.53×10^{-3}
Glycine	4.28×10^{-3}	3.23×10^{-3}
Urea	1.07×10^{-3}	1.09×10^{-3}
BMV	2.48×10^{-3}	6.69×10^{-4}
BMV alkali degradates	4.28×10^{-3}	1.63×10^{-4}
CIN acid degradates	2.81×10^{-3}	1.76×10^{-4}
Lidocaine HCl	2.20×10^{-2}	1.33×10^{-3}

[a]Each value is the average of three determinations.
[b]All interferents are in the form of 1×10^{-3} mol L^{-1} solution.

The constructed ISE selectivity coefficient ($K^{pot}_{A,B}$) was investigated with respect to CIN expected degradation products, structurally related compounds, and also versus several other interfering ions commonly used as pharmaceutical additives or that can be found in biological fluid.

Table III shows the potentiometric selectivity coefficients of the proposed sensors in the presence of some potentially interfering species, other local anesthetic (lidocaine hydrochloride), diluents, excipients (glucose and lactose) and some other inorganic cations (K^+, Na^+, and Ca^{2+}) that are usually found in biological fluids as well as both drug degradates. They reflect a very high selectivity of each of these electrodes for the CIN cation over most of the tested species. Overall, the designed electrodes are useful for the intended measurements. Clearly, the high selectivity of the proposed sensors toward CIN compared to other inorganic cations can be attributed to its higher lipophilicity, where Na^+ and K^+ did not interfere probably due to the difficulty of the ion exchange of these inorganic hydrophilic ions into the lipophilic membrane. Additionally, it is important to point out that the co-formulated drug, betamethasone valerate, did not interfere due to its poor water solubility. It is also obvious that the hydrolytic degradation products didn't show any significant interference with the parent drug. The most probable reason for selectivity is based mainly on the stereo specificity and electrostatic environment. It is dependent on the extent of fitting between the sites of the lipophilicity of the two competing species in the bathing solution side and the receptor of the ion- exchanger.[23] The observed selectivity facilitates the development of a CIN-ISE as a stability indicating method for determination of CIN.

In order to test the feasibility of analytical monitoring in real samples, the developed electrodes were employed to determine CIN concentrations in synthetic mixtures along with either its degradation products or co-formulated drug (BMV) and a commercially available pharmaceutical formulation containing CIN and BMV.

Synthetic mixture analysis.—In practical applications, primary and interfering ions are present at the same time and the electrode may behave quite differently in these cases as opposed when either type of ion is solely present. However, results obtained upon analysis of synthetic mixtures containing different ratios of intact drug with either its co-formulated drug (5:1) or its acid induced degradation products (100:0 to 20:80) showed that both sensors can be successfully used for selective determination of CIN in the presence of BMV and up to

Table IV. Determination of CIN in laboratory prepared mixtures containing different ratios of its acid degradation products by the proposed sensors.

Ratio %, Drug: degradate	Recovery (%) ± S.D[a] of CIN	
	Sensor 1 (liquid contact)	Sensor 2 (solid contact)
90: 10	98.59 ± 0.03	99.83 ± 1.56
70:30	99.71 ± 0.21	99.25 ± 0.51
50: 50	99.48 ± 0.02	100.42 ± 1.33
30: 70	99.93 ± 0.01	99.40 ± 0.31
20: 80	102.17 ± 0.67	101.49 ± 0.21

[a]Average of three determinations.

Table V. Determination of CIN in laboratory prepared mixtures containing different ratios of BMV by the proposed sensors.

Ratio %, CIN: BMV	Recovery (%) ± S.D[a] of CIN	
	Sensor 1 (liquid contact)	Sensor 2 (solid contact)
5: 1[b]	100.78 ± 0.09	100.02 ± 0.73
5.5:1	100.78 ± 0.37	99.79 ± 0.41
4.5: 1	101.01 ± 0.14	99.57 ± 0.73
5: 1.1	100.78 ± 0.09	100.08 ± 0.45
5: 0.9	100.34 ± 0.53	99.51 ± 0.49

[a]Average of three determinations.
[b]Dosage form ratio.

80% of its acid degradation products (Tables IV and V). Thus, they are recommended for use in stability-indicating methods.

Potentiometric determination of CIN in pharmaceutical formulation.—The proposed sensors were successfully employed for the selective determination of CIN in pharmaceutical formulation (Supraproct-S ointment). Susceptible ointment excipients did not show any interference. Thus, the determination of CIN was carried out with minimal treatment and without interference from BMV using sensors 1 and 2 (Table VI). Upon statistical comparison of the obtained results to those of the reported HPLC method,[5] no significant difference was observed (Table VI). Obviously, the potentiometric ISE method is straightforward, decrease analysis time, and thus reducing the cost per sample. Moreover, the electrochemical sensors are categorized by not only eco-friendly, but also, being relatively simple, sensitive and energy-saver.

Table VI. Determination of CIN in its pharmaceutical formulation by the suggested potentiometric procedure and reported method.

Dosage form	Sensor 1 (liquid contact)	Sensor 2 (solid contact)	Reported HPLC method[5a]
Mean ± SD[b]			
Supraproct -S ointment[c]	99.66 ± 0.66	99.00 ± 0.78	99.69 ± 1.19
% RSD[b]	0.66	0.79	1.19
Variance	0.44	0.61	1.42
Student's t-test[d]	0.05 (2.306)	1.09 (2.306)	—-
F-value[d]	3.23 (6.39)	2.33 (6.39)	—-

[a]Reported HPLC method using a C_{18} (250 cm × 4.6 mm, 5 μm) column, acetonitrile: acetate buffer (pH 6.5 ± 0.1) in a ratio of (55:45, v/v) as mobile phase, flow rate 1.2 mL/min and detection at 240 nm.
[b]Standard deviation and percentage relative standard deviation of five determinations for the proposed sensors and the reported HPLC method.
[c]each gram is labeled to contain 5 mg cinchocaaine hydrochloride and 1 mg betamethasone valerate, batch no: 0014.
[d]The values in the parenthesis are the corresponding theoretical values of t and F at P = 0.05 and n = 5.

Table VII. Determination of CIN in its pure form by the suggested potentiometric sensors and official method.

Value	Sensor 1 (liquid contact)	Sensor 2 (solid contact)	Official method[1a]
Mean ± SD[b]	99.93 ± 0.84	99.76 ± 1.13	100.05 ± 1.32
% RSD[b]	0.84	1.13	1.32
Variance	0.71	1.28	1.74
Student's t-test[c]	0.17 (2.306)	0.37 (2.306)	—-
F-value[c]	2.45 (6.39)	1.36 (6.39)	—-

[a]Titrimetric method against 0.1 M NaOH using a mixture of 0.01 M HCl and alcohol as a solvent with potentiometric detection of end point.
[b]Standard deviation and percentage relative standard deviation of five determinations for the proposed sensors and the official method.
[c]The values in the parenthesis are the corresponding theoretical values of t and F at P = 0.05 and n = 5.

Statistical comparison of the obtained results with reference method.—The results obtained were compared with those obtained by applying the official method[1] for pure CIN determination (Table VII). Statistical analysis of the results between the proposed and official method using student's t-test and F ratio showed there is no significant difference between them regarding accuracy and precision. This official titrimetric method involves the use of hazardous chemicals and solvents using a mixture of 0.01 M HCl and alcohol as a solvent with potentiometric detection of end point versus 0.1 M NaOH. A simple comparison between the proposed method and the official method can manifest that the proposed potentiometric method is a green environmentally friendly method that cut short time and effort.

Conclusions

A comparative potentiometric study between two types of electrodes (conventional, liquid inner contact and a glassy carbon) was constructed for determination of CIN. The sensors show favorable performance characteristics with short response times, low detection limits of 5.01×10^{-6} mol L^{-1} and 2.51×10^{-6} mol L^{-1} over the concentration range of 3.0×10^{-5} mol L^{-1} to 1.0×10^{-2} and 1.0×10^{-5} mol L^{-1} to 1.0×10^{-2} mol L^{-1}, for liquid contact and glassy carbon electrodes, respectively. Clearly, the glassy carbon sensor shows a lower detection limit due to its diminished current flux. The proposed sensors are sufficiently simple and compromise great advantages of fast response and eliminating any need for drug pre-treatment or separation steps. Furthermore, the described sensors are effectively used in the quantitative determination of CIN in presence of degradation products either in pure form or pharmaceutical formulations with BMV. Therefore, they can be used for routine analysis of CIN in quality-control laboratories. In general, the ISEs proposed here offered high simplicity in design and a very low limit of detection as well as being rapid, simple, and inexpensive and could compete with the many sophisticated methods currently available.

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