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Comparative study of 2-hydroxy propyl beta cyclodextrin and calixarene as ionophores in potentiometric ion-selective electrodes for neostigmine bromide

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ABSTRACT

Three novel neostigmine bromide (NEO) selective electrodes were investigated with 2-nitrophenyl octyl ether as a plasticiser in a polymeric matrix of polyvinyl chloride (PVC). Sensor 1 was fabricated using tetrakis(4-chlorophenyl)borate (TpClPB) as an anionic exchanger without incorporation of an ionophore. Sensor 2 used 2-hydroxy propyl β-cyclodextrin as an ionophore while sensor 3 was constructed using 4-sulfocalix-8-arene as an ionophore. Linear responses of NEO within the concentration ranges of 10^{-5} to 10^{-2}, 10^{-6} to 10^{-2} and 10^{-7} to 10^{-2} mol L^{-1} were obtained using sensors 1, 2 and 3, respectively. Nernstian slopes of 51.6 ± 0.8, 52.9 ± 0.6 and 58.6 ± 0.4 mV/decade over the pH range of 4–9 were observed. The selectivity coefficients of the developed sensors indicated excellent selectivity for NEO. The utility of 2-hydroxy propyl β-cyclodextrin and 4-sulfocalix[8]arene as ionophores had a significant influence on increasing the membrane sensitivity and selectivity of sensors 2 and 3 compared to sensor 1. The proposed sensors displayed useful analytical characteristics for the determination of NEO in bulk powder, different pharmaceutical formulations, and biological fluids (plasma and cerebrospinal fluid (CSF)) and in the presence of its degradation product (3-hydroxyphenyltrimethyl ammonium bromide) and thus could be used for stability-indicating methods.

1. Introduction

Neostigmine bromide [3-(dimethylcarbamoyloxy)-*N*,*N*,*N* trimethylanilinium bromide (mol. wt. 303.2)] is a quaternary amine that inhibits cholinesterase activity to prolong and intensify the muscarinic and nicotinic effects of acetylcholine. It is used in the treatment of myasthenia gravis, paralytic ileus, postoperative urinary retention and primary open-angle glaucoma [1]. Recently, neostigmine was investigated as an adjuvant to local anaesthetics and opioids for the relief of post-surgical pain after abdominal or gynaecological surgery via intrathecal and epidural injections [2].

Several methods have been reported for the determination of neostigmine bromide [NEO] in dosage forms and in biological fluids: potentiometric titration [3], spectrophotometry [4,5], thin-layer chromatography [6], high-performance liquid chromatography (HPLC) [7–9], gas-chromatography [10], electrophoresis [11,12] and voltammetry [13]. From these procedures, only the HPLC techniques [8,9] were recommended as stability-indicating assays and for determination of NEO in cerebrospinal fluid. Two ion selective electrodes have been reported for the determination of NEO [14,15] using a precipitation-based technique with Na tetraphenylborate as an anionic exchanger without incorporation of ionophores. These electrodes, however, did not examine the major degradation product of NEO (3-hydroxyphenyltrimethyl ammonium bromide) or drugs co-formulated with NEO, such as pilocarpine. Their selectivity coefficients were only tested for a few organic and inorganic cations.

Cyclodextrins are known to accommodate a wide variety of organic, inorganic and biologic guest molecules to form stable host–guest inclusion complexes or nanostructure supramolecular assemblies in their hydrophobic cavity while exhibiting high molecular selectivity and enantioselectivity [16,17]. They have been previously applied as sensor ionophores in potentiometric ion selective electrodes for the determination of fluorinated surfactants [18], chiral molecules incorporating aryl rings [19], protonated amines [20] and quaternary ammonium drugs [21].

Calixarenes are cavity-shaped cyclic oligomers made up of phenol units linked via alkylidene groups. Their configuration includes a number of selective factors, such as cavity-size, conformation and substituents, which leads to the formation of typical host–guest complexes with numerous compounds and allow for a variety of applications in ion-selective membranes and electrodes [22–24].

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The present work describes the use of functionalised cyclodextrin derivatives and sulphonated calix[8]arene as neutral ionophores for the development of novel sensors for the determination of NEO. These sensors were used for the determination of NEO in bulk powder, different pharmaceutical formulations, and biological fluid (plasma and CSF) and in the presence of its degradation product.

2. Experimental

2.1. Apparatus

A Jenway digital ion analyser model 3330 (Essex, UK) with Ag/AgCl double junction reference electrode no. 924017-LO-Q11C was used for potential measurements. A Jenway pH glass electrode no. 924005-BO3-Q11C (Essex, UK) and a Bandelin sonorox magnetic stirrer model Rx 510 S (Budapest, Hungary) were used for pH adjustments.

2.2. Chemicals and reagents

Neostigmine bromide, 99.69%, was obtained from Sigma (St. Louis, USA). Amostigmine® tablets (15 mg/tablet, Amoun Pharmaceutical Co., Cairo, Egypt), Amostigmine® injection (0.5 mg/mL, Amoun Pharmaceutical Co., Cairo, Egypt) and Normastigmine *mit* pilocarpine eye drops® (mixture of 30 mg neostigmine bromide and 20 mg pilocarpine hydrochloride in 1 mL, Sigmapharm, Vienna, Austria) were used in this work.

All chemicals and reagents used were of analytical reagent grade, and water was bi-distilled. Polyvinyl chloride (PVC), 2-hydroxy propyl β-cyclodextrin and 4-sulfocalix[8]arene were obtained from Fluka (Steinheim, Germany). 2-Nitrophenyl octyl ether (NPOE) and tetrakis(4-chlorophenyl)borate (TpClPB) were purchased from Aldrich (Steinheim, Germany). Tetrahydrofuran (THF) was obtained from BDH (Poole, England). Potassium chloride and ammonium sulphate were obtained from Prolabo (Pennsylvania, USA). Britton–Robinson buffer (BRB) (pH 2–12) was prepared by mixing different volumes of 0.04 $\mathrm{mol\,L^{-1}}$ acetic acid, 0.04 $\mathrm{mol\,L^{-1}}$ phosphoric acid, 0.04 $\mathrm{mol\,L^{-1}}$ boric acid and 0.2 $\mathrm{mol\,L^{-1}}$ sodium hydroxide. Plasma and cerebrospinal fluid were supplied by VACSERA (Giza, Egypt) and used within 24 h.

2.3. Procedures

2.3.1. Fabrication of membrane sensors

For the preparation of sensor 1, 400 mg NPOE was mixed with 50 mg TpClPB and 190 mg PVC in a 5-cm Petri dish. The mixture was dissolved in 6 mL THF. Fifty milligrams of 2-hydroxy propyl β-cyclodextrin or 50 mg 4-sulfocalix-8-arene were added to the previous components for the preparation of sensors 2 and 3, respectively. The Petri dishes were covered with filter paper and left to stand overnight at room temperature to allow solvent evaporation.

Master membranes 0.1 mm in thickness were obtained. From each master membrane, a disk (about 8 mm in diameter) was cut using a cork borer and pasted using THF to an interchangeable PVC tip that was clipped into the end of an electrode glass body. The electrodes were then filled with an internal solution of equal volumes of 10^{-2} $\mathrm{mol\,L^{-1}}$ NEO and 10^{-2} $\mathrm{mol\,L^{-1}}$ KCl. Ag/AgCl wire (1 mm diameter) was used as an internal reference electrode. The sensors were conditioned by soaking in 10^{-2} $\mathrm{mol\,L^{-1}}$ aqueous NEO solution for 24 h, and they were stored in the same solution when not in use.

2.3.2. Sensors calibration

The conditioned sensors were calibrated by separately transferring 50 mL aliquots of solutions (10^{-7} to 10^{-2} $\mathrm{mol\,L^{-1}}$) of NEO into a series of 100-mL beakers. The membrane sensors, in conjunction with Ag/AgCl reference electrode, were immersed in the above test solutions and allowed to equilibrate while stirring. The potential was recorded after stabilising to ±1 mV, and the electromotive force was plotted as a function of the negative logarithm of NEO concentration. The above procedure was repeated after adding 2 mL of 2 $\mathrm{mol\,L^{-1}}$ $(NH_4)_2SO_4$ (ionic strength adjustor) to the measured solutions.

2.3.3. Effect of pH

The effect of pH on the response of the investigated electrodes was studied using 10^{-3} and 10^{-4} $\mathrm{mol\,L^{-1}}$ solutions of NEO in BRB with pH ranging from 2 to 10.

2.3.4. Sensors selectivity

The potentiometric selectivity coefficients ($K_{A.B}^{pot}$) of the proposed sensors towards different substances were determined by a separate solution method using the following equation [25]:

$$-\log(K_{A.B}^{pot}) = \frac{E_1 - E_2}{2.303\,RT/Z_AF} + \left(1 - \frac{Z_A}{A_B}\right)\log a_A$$

where $K_{A.B}^{pot}$ is the potentiometric selectivity coefficient, E_1 is the potential measured in 10^{-3} $\mathrm{mol\,L^{-1}}$ NEO solution, E_2 is the potential measured in 10^{-3} $\mathrm{mol\,L^{-1}}$ interferent solution, Z_A and Z_B are the charges of NEO and interfering ion, respectively, a_A is the activity of the drug and $2.303RT/Z_AF$ represents the slope of the investigated sensors (mV/concentration decade).

2.3.5. Determination of NEO in pharmaceutical preparations

A portion of Amostigmine® tablets powder equivalent to 0.0303 g NEO, 5.0 mL Amostigmine® injection and 1.0 mL Normastigmine *mit* pilocarpine® eye drops were transferred separately into three 50-mL volumetric flasks and filled to the mark with bi-distilled water or BRB solution at pH 7. The concentrations of these prepared samples were 2.0×10^{-3}, 1.6×10^{-4} and 1.9×10^{-3} $\mathrm{mol\,L^{-1}}$, respectively. 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.

2.3.6. Determination of NEO in the presence of its alkaline degradate

A degraded sample of NEO was prepared by adding 5 mL NaOH (0.1 $\mathrm{mol\,L^{-1}}$) to 10 mL drug solution (10^{-2} $\mathrm{mol\,L^{-1}}$) and refluxing for 10 min. The resulting solution was tested for complete degradation by the thin layer chromatography technique using butanol:methanol:H_2O:NH_3 (5:4:1:0.01 ratio by volume) as a mobile phase and detecting the spots at 254 nm. The degraded solution was neutralised, transferred quantitatively into a 100-mL volumetric flask and brought to volume with deionised water. Aliquots of standard drug solution (10^{-3} $\mathrm{mol\,L^{-1}}$) were mixed with its degraded sample (10^{-3} $\mathrm{mol\,L^{-1}}$) in different ratios. The emf values of these laboratory-prepared mixtures were recorded and results were compared with the calibration plot.

2.3.7. Determination of NEO in plasma

One millilitre of each of 10^{-2}, 10^{-3} and 10^{-4} $\mathrm{mol\,L^{-1}}$ standard drug solution were added separately into three 20-mL stoppered shaking tubes each containing 9 mL of plasma and the tubes were shaken for 1 min. The membrane sensors were immersed in conjunction with the reference electrode in these solutions and then washed with water between measurements. The emf produced for each solution was measured by the proposed sensors, and the concentration of NEO was determined from the corresponding regression equation.

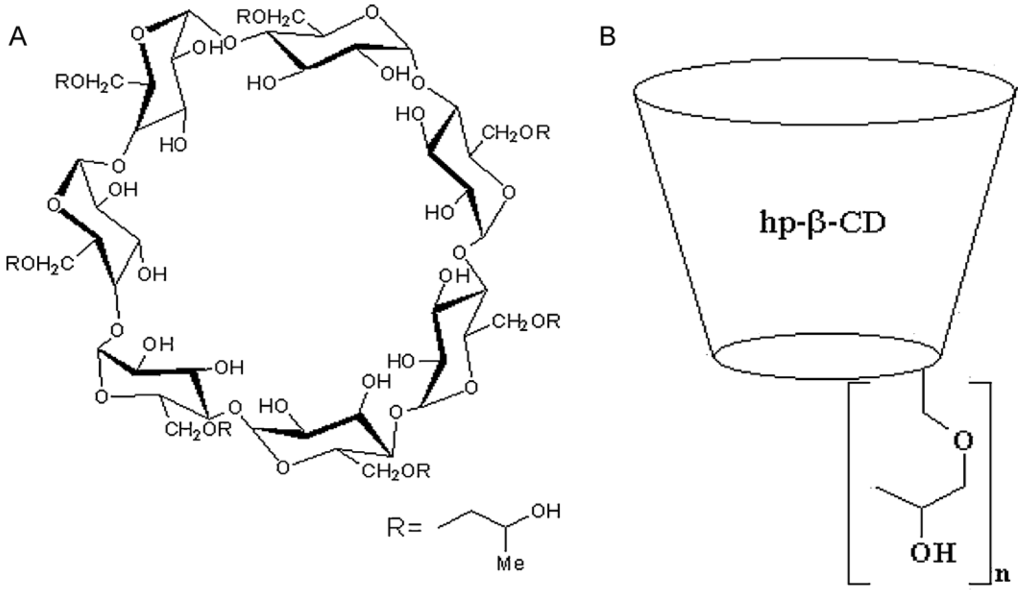

Fig. 1. Chemical structure (A) and toroidal shape (B) of the 2-hydroxy propyl β-cyclodextrin molecule.

2.3.8. Determination of NEO in CSF

One millilitre of 10^{-2} and of 10^{-3} mol L^{-1} NEO solution were added separately to two 25-mL beakers. Each contained 9 mL of CSF, and the beakers were vortexed for 1 min. The membrane sensors were immersed in conjunction with the reference electrode in these solutions and then washed with water between measurements. The emf produced for each solution was measured by the proposed sensors, and then the concentration of NEO was determined from the corresponding regression equation.

3. Results and discussion

The molecular recognition and inclusion complexation are of current interest in host–guest and supramolecular chemistry and offer a promising approach to chemical sensing [26,27]. The use of selective inclusion complexation and complementary ionic or hydrogen bonding are two main strategies for preparing synthetic host molecules, which recognise the structure of guest molecules [28].

Modified cyclodextrins (CDs), either natural or synthetic, are viewed as molecular receptors, as is shown in Fig. 1. In the case of natural CD, cooperative binding with certain guest molecules was mostly attributed to intermolecular hydrogen bonding between the CD molecules, while intermolecular interactions between the host and guest molecules (hydrogen bonds, hydrophobic interactions and Van der Waals forces) contributed to cooperative binding processes when synthetic CDs were used [29]. Although the size and geometry of the guest mainly govern the binding strength, it is possible to influence the host–guest interactions by modifying the three hydroxyl groups on each glucose unit. Indeed, the use of 2-

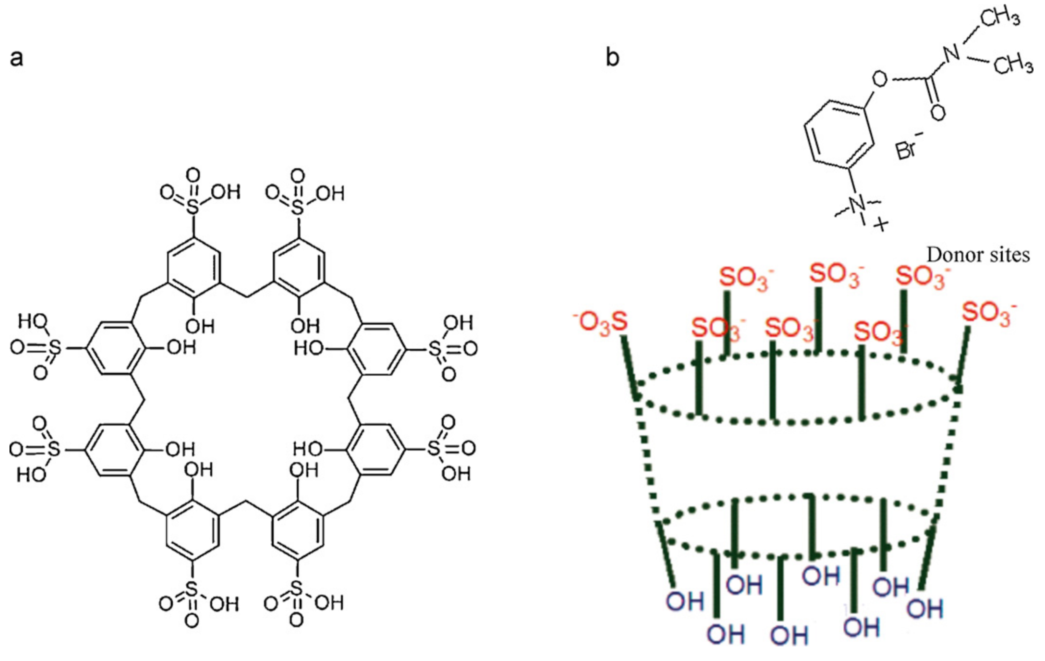

Fig. 2. Chemical structure of the 4-sulfocalix[8]arene molecule (A). Mode of attachment between the sulphonic acid groups of 4-sulfocalix-8-arene and NEO (B).

Table 1
Electrochemical response characteristics of the three investigated NEO sensors.

Parameter	Sensor 1	Sensor 2	Sensor 3
Slope (mV/decade)[a]	51.6	52.9	58.6
Intercept (mV)	100.7	416.2	266.8
LOD (mol L^{-1})[b]	7.8×10^{-6}	2.4×10^{-7}	3.8×10^{-8}
Response time (s)	35	15	10
Working pH range	4–9	4–9	4–9
Concentration Range (mol L^{-1})	10^{-5} to 10^{-2}	10^{-6} to 10^{-2}	10^{-7} to 10^{-2}
Stability (days)	16	44	56
Average recovery (%) ± S.D.[a]	99.11 ± 1.223	100.74 ± 0.921	99.54 ± 0.841
Correlation coefficient	0.9997	0.9999	0.9999
Ruggedness[c]	98.44	99.41	99.72

[a] Average of five determinations.
[b] Limit of detection (measured by interception of the extrapolated arms of Fig. 3).
[c] Average recovery percent of determining 10^{-3} and 10^{-4} mol L^{-1} NEO for the studied electrodes using Jenway 3510 digital ion analyser instead of the 3310 model.

hydroxy propyl β-cyclodextrin enhanced the interaction properties between host and guest molecules [30].

Calixarenes are well-known as selective ligands for various ions through dipole–dipole interactions, as shown in Fig. 2. They can complex with a large variety of cation substrates to form stable host–guest inclusion complexes. This property of calixarenes has been largely exploited for the development of a number of cation-selective electrodes [31–33].

The present work evaluates the possibility of using 2-hydroxy propyl β-cyclodextrin and sulphonated calix[8]arene as sensor ionophores in the preparation of NEO-selective electrodes 2 and 3, respectively, using PVC as a polymeric matrix to immobilise the sensors and to attain the formation of highly stable complexes.

3.1. Performance characteristics of NEO sensors

The positive NEO ion prefers the high donation sites (OH-groups and sulphonic acid) of 2-hydroxy propyl β-cyclodextrin and calixarene structures rather than the methyl groups. Thus, in the absence of ionophores in sensor 1, the lowest slope value is found accompanied by the highest selectivity coefficient values. A higher selectivity coefficient value corresponds with more attack by interfering cations on the electrode membrane.

The presence of OH-groups only in sensor 2 was not enough to perform the proper chelation, which was demonstrated by a slope of 52.3 mV/decade and the high selectivity coefficient values compared to sensor 3.

The sulphonated calix[8]arene-based sensor 3 shows the best Nernstian slope (58.6 mV/decade) and selectivity coefficient values. The host–guest complex is stabilised via an electrostatic interaction between the cationic NEO and anionic sulphonated calix[8]arene. Moreover, calix[8]arene has a larger internal cavity size (9.5 Å) [34] than 2-hydroxy propyl β-cyclodextrin (6 Å) [35]. This allows the drug to fit well in the calixarene cavity and strongly bond to the calixarene donation sites.

The results reveal that, as ionophores, 2-hydroxy propyl β-cyclodextrin and calix-8-arene provide high stability to the complexes formed with cationic drug present in solution; thus, the membrane selectivity and sensitivity are substantially enhanced.

The electrochemical performance characteristics of the proposed sensors were systematically evaluated according to IUPAC standards [25]. Table 1 shows the results obtained over a period of two months for two different assemblies of each sensor. The addition of ionic strength adjustor to different concentrations of NEO solutions prepared for calibration plots shows no significant differences on the resulting accuracy of the proposed sensors. This is in agreement with previous references [15,33], which avoid the use of ionic strength adjustor. Typical calibration plots are shown in Fig. 3.

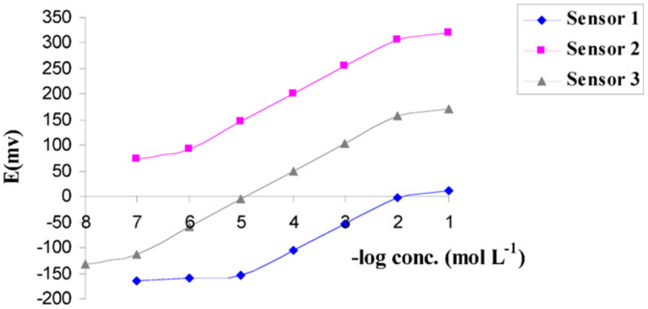

Fig. 3. Profile of the potential in mV versus −log concentrations of NEO in mol L^{-1} obtained with sensors 1, 2 and 3.

The slopes of the calibration plots are 51.6, 52.9 and 58.6 mV/concentration decade for sensors 1, 2 and 3, respectively. Deviation from the ideal Nernstian slope (60 mV) is due to the electrodes responding to the activity of the drug cation rather than its concentration.

The sensors displayed constant potential readings for day to day measurements, and the calibration slopes did not change by more than ±2 mV/decade over a period of 16, 44 and 56 days for sensors 1, 2 and 3, respectively. The detection limits of the three sensors were estimated according to the IUPAC definition [25]. Table 1 shows that sensor 3 can detect NEO in very dilute solutions down to 3.8×10^{-8} mol L^{-1}. This agrees with the idea that NEO is typically bonded with the very polar sulphonic acid groups.

3.2. Dynamic response time

Dynamic response time is an important factor for analytical applications of ion-selective electrodes. In this study, practical response time was recorded by increasing NEO concentration by up to 10-fold. The required time for the sensors to reach values within ±1 mV of the final equilibrium potential was 35, 15 and 10 s for sensors 1, 2 and 3, respectively.

3.3. Effect of pH and temperature

For quantitative measurements with ion selective electrodes, studies were carried out to reach the optimum experimental conditions. The potential pH profile obtained indicates that the responses of the three sensors are fairly constant over the pH range 4–9. Therefore, the pH range from 4 to 9 was assumed to be the working pH range of the three sensors.

The results suggest that the electrodes exhibit a slight increase in their potential as the temperature rises in the range of 20–35 °C. However, the calibration plots obtained at different temperatures are parallel, and the limit of detection, slope and response time do not significantly vary with temperature indicating reasonable thermal stability of PVC membranes up to 35 °C.

3.4. Sensors selectivity

Table 2 shows the potentiometric selectivity coefficients of the proposed sensors in the presence of degradates, co-formulated drug (pilocarpine), other anticholinesterase drugs and some other inorganic cations (K^+, Na^+, $NH_4{}^+$, Mg^{++}, and Ca^{++}) that are usually found in biological fluids. The results reveal that the proposed membrane sensors display high selectivity and that sensors 2 and 3 are at least 10–100 times more selective than sensor 1. Sensor 3 displays higher selectivity and lower response for the potentially interfering species and co-formulated drugs (pilocarpine) than sensor 2. This can be attributed to the preferential interaction between

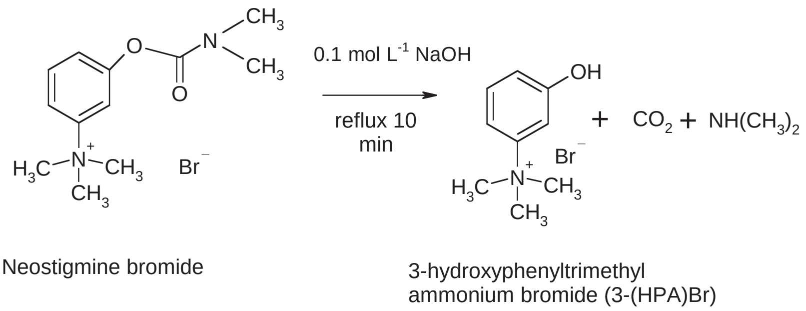

Fig. 4. Alkaline degradation of neostigmine bromide [37].

Table 2
Potentiometric selectivity coefficients (K_I^{pot}) of the three proposed sensors using the separate solutions method (SSM) [25].

Interferent[b]	Selectivity coefficient[a]		
	Sensor 1	Sensor 2	Sensor 3
Degradate[c]	3.2×10^{-1}	8.2×10^{-3}	8.3×10^{-4}
Pyridostigmine bromide	4.8×10^{-1}	8.5×10^{-2}	7.2×10^{-3}
Distigmine bromide	6.4×10^{-2}	7.8×10^{-3}	6.3×10^{-4}
Rivastigmine hydrogen tartrate	8.3×10^{-1}	8.1×10^{-3}	3.7×10^{-4}
Physostigmine salicylate	4.7×10^{-1}	2.9×10^{-3}	7.1×10^{-4}
Glycine	5.2×10^{-1}	1.3×10^{-2}	1.9×10^{-3}
NaCl	3.2×10^{-2}	7.2×10^{-4}	8.9×10^{-4}
KCl	1.9×10^{-2}	6.3×10^{-4}	9.6×10^{-4}
$CaCl_2$	8.8×10^{-2}	9.4×10^{-3}	7.7×10^{-4}
$MgCl_2$	5.5×10^{-2}	2.3×10^{-3}	1.8×10^{-4}
$(NH_4)_2SO_4$	4.1×10^{-3}	6.9×10^{-3}	8.1×10^{-4}
Pilocarpine HCl	2.2×10^{-1}	4.9×10^{-3}	2.1×10^{-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.
[c] 3-Hydroxyphenyltrimethyl ammonium bromide.

the NEO cation and very polar sulphonic acid groups present in the calix[8]arene structure.

3.5. Potentiometric determination of NEO in pharmaceutical formulations

The proposed sensors were applied for the analysis of NEO pharmaceutical formulations in aqueous and buffered solutions. The results show no significant differences between these two cases. This can be ascribed to the wide working pH range from the acidic side (pH 4) to the basic side (pH 9) of the proposed sensors. The results also prove the applicability of the three sensors for the determination of pharmaceutical formulations containing NEO alone. However, results show that pilocarpine interferes with NEO measurements using sensor 1 and that, therefore, the use of this sensor is not recommended in the presence of pilocarpine. These data are shown in Table 3.

Table 4
Determination of NEO in laboratory prepared mixtures containing different ratios of NEO and its induced alkaline degradation product by the proposed sensors.

Ratio (%) drug:degradate	Drug recovery (%) ± S.D.[a]		
	Sensor 1	Sensor 2	Sensor 3
100:0	98.62 ± 0.93	98.12 ± 0.89	99.32 ± 0.78
90:10	98.52 ± 0.89	98.96 ± 0.98	99.46 ± 0.68
80:20	99.11 ± 0.87	98.52 ± 0.89	98.52 ± 0.89
70:30	107.4 ± 1.36	100.49 ± 0.97	100.41 ± 0.97
60:40	114.3 ± 1.79	100.68 ± 1.09	100.68 ± 0.72
50:50	121.53 ± 1.99	100.91 ± 1.16	100.91 ± 0.86
40:60	129.51 ± 1.97	99.53 ± 1.73	99.53 ± 0.63
30:70	142.19 ± 1.08	98.01 ± 1.09	99.09 ± 1.21
20:80	158.13 ± 1.69	98.52 ± 1.81	99.42 ± 1.29
10:90	187.56 ± 1.88	97.13 ± 1.88	100.23 ± 1.08

[a] Average of three determinations.

To examine the validity of the proposed sensors, the obtained results were compared to those of the U.S. Pharmacopeia (USP) method [36] and no significant difference was observed. Moreover, the proposed sensors do not require preliminary drug extraction as described in the USP method.

3.6. Potentiometric determination of NEO in the presence of its alkaline degradate

Complete degradation of NEO was induced by boiling with 0.1 mol L^{-1} NaOH for 10 min. Fig. 4 shows the reported alkaline degradation of the drug [37]. The degradation products are 3-hydroxyphenyltrimethyl ammonium bromide, carbon dioxide and dimethylamine. The induced alkaline degradation was tested by TLC, and complete separation, with R_f values of 0.31 for NEO and 0.79 for its degradation product, was obtained. Table 4 shows the results obtained upon analysis of synthetic mixtures containing different ratios of intact drug and degraded sample varying from 100:0 to 10:90. The results shows that sensors 2 and 3 can be successfully

Table 3
Determination of NEO in different pharmaceutical formulations by the three proposed electrodes and the official method [36].

Pharmaceutical formulations	Recovery (%) ± S.D.[a] of NEO			
	Sensor 1	Sensor 2	Sensor 3	Official method[b]
Amostigmine® injection (0.5 mg/mL)	100.98 ± 0.92	98.08 ± 0.65	99.81 ± 0.78	98.42 ± 1.44
t-Test[c]	1.293 (2.306)	0.456 (2.306)	0.258 (2.306)	
F[c]	3.18 (6.39)	1.88 (6.39)	1.28 (6.39)	
Amostigmine® tablets (15 mg/tablet)	100.53 ± 1.32	99.12 ± 0.45	100.73 ± 0.97	98.18 ± 1.97
t-Test[c]	1.144 (2.306)	0.866 (2.306)	1.278 (2.306)	
F[c]	2.33 (6.39)	1.13 (6.39)	2.08 (6.39)	
Normastigmine® *mit* pilocarpine eye drops 30 mg NEO and 20 mg PILO/1 mL	142.99 ± 1.97	101.13 ± 0.79	98.53 ± 0.77	

[a] Average of five determinations.
[b] Spectrophotometric measurement at 420 nm using hexanitrodiphenylamine in methylene chloride.
[c] The values in parentheses are the corresponding theoretical values for *t* and *F* at $P = 0.05$.

Table 5
Determination of NEO in spiked human plasma by the proposed sensors.

Added (μg/ml)	Recovery (%) ± S.D.[a]		
	Sensor 1	Sensor 2	Sensor 3
10^{-3} (303.2)	98.98 ± 1.39	98.17 ± 0.96	99.11 ± 0.83
10^{-4} (30.32)	99.01 ± 1.27	98.11 ± 1.13	99.32 ± 0.87
10^{-5} (3.032)	–	101.45 ± 1.56	98.19 ± 1.27

[a] Average of three determinations.

Table 6
Determination of NEO in spiked cerebrospinal fluid by the proposed sensors.

	Recovery (%) ± S.D.[a]		
Added (μg/ml)	Sensor 1	Sensor 2	Sensor 3
10^{-3} (303.2)	97.98 ± 1.88	98.17 ± 1.66	99.11 ± 0.83
10^{-4} (30.32)	99.09 ± 1.95	98.11 ± 1.13	98.52 ± 0.97

[a] Average of three determinations.

used for selective determination of intact drug in the presence of >90% of its degradate. Sensor 1 suffers from high interference when the degradate concentration reaches about 30%. Thus, sensors 2 and 3 are recommended for use in stability-indicating methods.

3.7. Potentiometric determination of NEO in plasma and CSF

The results obtained for the determination of NEO in spiked human plasma show that a wide concentration range of the drug can be determined by the investigated sensors with high precision and accuracy. The results presented in Table 5 show that sensors 2 and 3 are more sensitive than sensor 1 in plasma samples.

For the application to CSF, it was found that the three sensors are reliable and give stable results with very good accuracy and high percentage recovery without preliminary extraction procedures, which is shown in Table 6. The pH of these samples was measured before spiking and was found to be 7 ± 0.5, which is within the pH working range of the proposed sensors.

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

4. Conclusion

The described sensors are sufficiently simple and selective for the quantitative determination of NEO in pure form, pharmaceutical formulations, in the presence of its degradate and in plasma and CSF. The use of 2-hydroxy propyl β-cyclodextrin and sulphonated calix-8-arene as ionophores increased the membrane sensitivity and selectivity of sensors 2 and 3 in comparison with sensor 1. The proposed sensors offer advantages of fast response and elimination of drug pre-treatment or separation steps. They can therefore be used for routine analysis of NEO in quality-control laboratories.

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