Scientific paper

Ion-Selective Electrodes for the Potentiometric Determination of Pramoxine HCl Using Different Ionophores

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Received: 07-04-2012

Abstract

Four novel pramoxine HCl (PAM) selective electrodes were investigated with 2-nitrophenyl octylether as a plasticiser in a polymeric matrix of polyvinyl chloride (PVC). Sensor 1 was fabricated using sodium-tetraphenylborate (TPB) as an anionic exchanger without incorporation of an ionophore. Sensor 2 used 2-hydroxy propyl -cyclodextrin as an ionophore, while sensors 3 and 4 were constructed using 4-sulfocalix-6-arene and 4-sulfocalix-8-arene respectively as ionophores. Linear responses of PAM within the concentration ranges of 1.0×10^{-4} to 1.0×10^{-2} mol L^{-1} and 1.0×10^{-5} to 1.0×10^{-2} mol L^{-1} were obtained using sensors 1 and 2, respectively and 1.0×10^{-6} to 1.0×10^{-2} mol L^{-1} were obtained using sensors 3 and 4. Nernstian slopes of 50.4 ± 0.6, 54.3 ± 0.8, 56.3 ± 0.3 and 59.1 ± 0.5 mV/decade over the pH range of 3.0–6.0 were observed. The selectivity coefficients of the developed sensors indicated excellent selectivity for PAM. The utility of 2-hydroxy- propylcyclodextrin (2HP-β-CD) and 4-sulfocalix [6,8] arene (SC 6, 8) as ionophores had a significant influence on increasing the membrane sensitivity and selectivity of sensors 2, 3 and 4 compared to sensor 1. The proposed sensors displayed useful analytical characteristics for the determination of PAM in bulk powder, pharmaceutical formulation, and in biological fluid. Validation of the method showed the suitability of the proposed electrodes for the use in the quality control assessment of the drug. Furthermore, statistical comparison between the results obtained by the proposed method and the official method of the drug was performed and no significant difference was found.

Keywords: Pramoxine HCl, Pramocaine HCl, 2-Hydroxy propyl-β-cyclodextrin, Calixarene, Potentiometry, Plasma

1. Introduction

Pramoxine HCl (PAM), 4-[3-(4-Butoxphenoxy) propyl] morpholine hydrochloride (Fig. 1).[1]

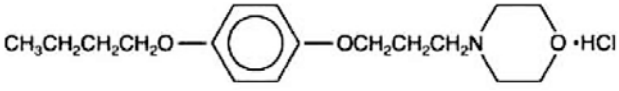

Fig. 1: structure of Pramoxine hydrochloride $C_{17}H_{27}NO_3$ HCl; mol. wt.: 329.87

It is a local anaesthetic used for surface anaesthesia. It is used alone or with corticosteroids and other drugs, usually in a concentration of 1%, in a wide range of formulations for the relief of pain and itching associated with minor skin conditions and anorectal disorders. Initial burning or stinging may occur following topical application.[1] Literature survey represented below reveals different analytical methods for quantification of the drug in pure form, in pharmaceutical dosage form and in biological fluids

Method	Reference number
HPLC	2–8
TLC	9
GC	9

In fact now electroanalytical methods are in a position to challenge chromatographic methods when concerned biological fluids or pharmaceutical dosage forms.[10–26]

Electro-analytical methods are clean and sensitive methods for the quantification of pharmaceuticals.[27–41] Therefore, the aim of our work is to develop sensitive,

clean, simple, accurate, rapid and precise electroanalytical method for the determination of PAM in the presence of hydrocortisone acetate.

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 highmolecular selectivity and enantioselectivity.[42,43] They have been previously applied as sensor ionophores in potentiometric ion selective electrodes for the determination of fluorinated surfactants,[44] chiral molecules incorporating aryl rings,[45] protonated amines[46] and quaternary ammonium drugs.[47] 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 substituent 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.[48–53]

The present work describes the use of functionalized cyclodextrin derivatives and sulphonated calix [6,8] arene as neutral ionophores for the development of novel sensors for the determination of PAM. These sensors were used for the determination of PAM in bulk powder, pharmaceutical formulation, and in biological fluid (plasma).

2. Experimental

2. 1. Apparatus

Jenway digital ion analyzer Model 3330 (Spectronic Camspec Ltd, Garforth, UK) with Ag/AgCl double junction reference electrode No. Z113107-1EAPW (Aldrich Chemical Co., St. Louis, MO) was used. The influence of pH on the response of the electrodes was studied using a glass pH electrode (Jenway, Essex, UK No. 924005-BO3-Q11C). Samples were stirred using a magnetic stirrer (Bandelin Sonorox, Rx510S, Budapest, Hungary).

2. 2. Chemicals and Reagents

All chemicals and solvents used during the synthesis of the sensors and all over the potentimetric determination of the drug were handled in the fuming cupboard, wearing gloves and masks and the measure of the solvents was done using pipettes with the aid of pipette filler.

Pramoxine HCl (PAM) – Batch number 10020301 was obtained from Sigma Pharmaceutical industries, Cairo, Egypt and its purity was found to be 100.1 ± 0.6% according to US pharmacopeial method,[2] to have purity. Pramacort cream-Batch number 92308 was obtained from Sigma Pharmaceutical industries, Cairo, Egypt and it was labeled to contain 1% pramoxine HCl and 2.5% hydrocortisone acetate. All chemicals and reagents used were of analytical reagent grade, and water was bi-distilled. Polyvinyl chloride (PVC), 2-hydroxy propyl-β-cyclodextrin (2HP-β-CD) and 4-sulfocalix [6, 8] arene (SC6 or SC8) were obtained from Fluka (Steinheim, Germany). 2-Nitrophenyl octylether (NPOE) and sodium tetraphenylborate (TPB) were purchased from Aldrich (Steinheim, Germany). Tetrahydrofuran (THF) was obtained from BDH (Poole, England). Potassium chloride was obtained from Prolabo (Pennsylvania, USA). Acetate buffer pH 5 (prepared by mixing 70 ml of 0.2 M sodium acetate and 30 ml of 0.2 M acetic acid),[54] sodium acetate and acetic acid were obtained from Prolabo, (Pennsylvania, USA). Plasma fluid was supplied by VACSERA (Giza, Egypt).

2. 3. Procedures

2. 3. 1. Fabrication of Membrane Sensors

For the preparation of sensor 1, a portion equivalent to 0.4 g NPOE was mixed with 0.05 g TPB and 0.19 g PVC in a 5-cm Petri dish. The mixture was dissolved in 6 ml THF. Portions equivalent to 0.05 grams of 2HP-β-CD, 0.05g of SC6 or SC8 were added to the previous components for the preparation of sensors 2, 3 and 4, 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 1.0×10^{-2} mol L^{-1} PAM and 1.0×10^{-2} mol L^{-1} KCl. Ag/AgCl wire (1 mm diameter) was used as an internal reference electrode. The sensors were conditioned by soaking in 1.0 10^{-2} mol L^{-1} aqueous PAM 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 (1.0×10^{-6} to 1.0×10^{-2} mol L^{-1}) of PAM 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 stabilizing to ±1 mV, and the electromotive force was plotted as a function of the negative logarithm of PAM concentration.

2. 3. 3. Effect of pH

The effect of pH on the response of the investigated electrodes was studied using 1.0×10^{-3} and 1.0×10^{-4} mol L^{-1} solutions of PAM in pH ranging from 1 to 11 by adding drops of 1 M HCl and 1 M NaOH. The potentials obtained at each value were recorded.

2. 3. 4. Sensors Selectivity

The potentiometric selectivity coefficients ($K^{pot}_{PAM.I}$) of the proposed sensors towards different substances were determined by a separate solution method using the following equation.[55]

$-\log (K^{pot}_{PAM.I}) = E_1 - E_2/2.303\ RT/Z_{PAM}F + (1 - Z_{PAM}/Z_I) \log \alpha_{PAM}$ where $K^{pot}_{PAM.I}$ is the potentiometric selectivity coefficient, E_1 is the potential measured in 1.0×10^{-3} mol L^{-1} PAM solution, E_2 is the potential measured in 1.0×10^{-3} mol L^{-1} interferent solution, Z_{PAM} and Z_I are the charges of PAM and interfering ion, respectively, α_{PAM} is the activity of the drug and $2.303RT/Z_{PAM}F$ represents the slope of the investigated sensors (mV/concentration decade).

2. 3. 5. Determination of PAM in Pharmaceutical Preparations

Two portions of pramacort cream equivalent to 0.0329 g and 0.00329 g PAM were accurately transferred into two 100-ml beakers. Then thirty ml acetate buffer was added, and left for heating till complete homogenization. The homogenized solutions were accurately transferred to two 100-mL volumetric flasks and completed to the mark with acetate buffer at pH 5. The concentrations of the prepared samples were 1.0×10^{-3} and 1.0×10^{-4} mol L^{-1}. 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 PAM in Plasma

Aliquots equivalent to one ml of 1.0×10^{-2}, 1.0×10^{-3} and 1.0×10^{-4} mol L^{-1} standard drug solution were added separately into three 20-ml stoppered shaking tubes each containing 9 ml of plasma. The tubes were shaken for 1 min. the first two concentrations were measured by sensors 1, 2, 3 and 4 while the third was measured by sensors 2, 3 and 4. The membrane sensors were immersed in conjunction with the reference electrode in these solutions and then washed with acetate buffer between measurements. The emf produced for each solution was measured by the proposed sensors and the concentration of PAM was determined from the corresponding regression equations.

2. 3. 7. Comparison with the Official Method

PAM was also determined by the official method of the drug. The official method of PAM[2] used the high performance liquid chromatographic technique on a column (4.6 mm × 25 cm containing packing L1), the mobile phase consisted of acetonitile and pH 7.5 phosphate buffer (55:45), detection was performed using a UV detector setting at 224 nm and the flow rate was 2 ml/min. Statistical comparison between the results obtained by the proposed method and those obtained by official method for PAM was done.

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.[56,57] The use of selective inclusion complexation and complementary ionic or hydrogen bonding are two main strategies for preparing synthetic host molecules, which recognize the structure of guest molecules.[58] Modified cyclodextrins (CDs), either natural or synthetic, are viewed as molecular receptors, as is shown in Fig. 2. 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.[59] 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 glu-

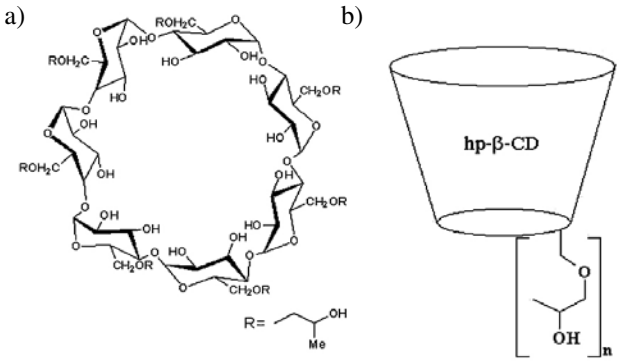

Fig. 2. Chemical structure (a) and toroidal shape (b) of the 2-hydroxy propyl β-cyclodextrin molecule.

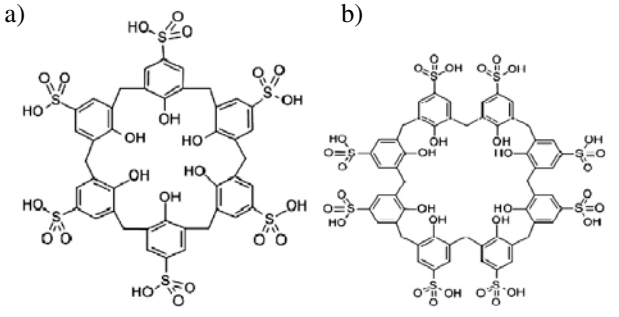

Fig. 3. Chemical structure of the 4-sulfocalix[6]arene molecule (A) and 4-sulfocalix[8]arene molecule (B)

cose unit. Indeed the use of 2-hydroxy propyl β-cyclodextrin enhanced the interaction properties between host and guest molecules.[60] Calixarenes are well-known as selective ligands for various ions through dipole–dipole interactions, as shown in Fig. 3. 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.[61–63]

The present work evaluates the possibility of using 2-hydroxypropyl-β-cyclodextrin, sulphonated calyx[6] arene and sulphonated calyx[8]arene as sensor ionophores in the preparation of PAM-selective electrodes 2,3 and 4, respectively, using PVC as a polymeric matrix to immobilize the sensors and to attain the formation of highly stable complexes.

3. 1. Performance Characteristics of PAM Sensors

The positive PAM 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.[62] Thus, in the absence of ionophores in sensor 1, the lowest slope value (50.4 mV/decade) 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 54.3 mV/decade and the high selectivity coefficient values compared to sensors 3 and 4.[64] The sensors 3 and 4 that are based on using sulphonated calyx[6,8]arene as an ionophore show the best Nernstian slopes (56.3 and 59.1 mV/decade respectively) and selectivity coefficient values. The host–guest complex is stabilized via an electrostatic interaction between the cationic PAM and anionic sulphonated calix[6,8]arene. The higher the anionic sulphonated groups and the larger internal cavity size in sensor 4, the higher the stability and the best Nernstian slope (59.1 mV/decade) than in sensor 3 which shows a slope value (56.3 mV/decade).[64] Moreover, calix[6,8]arene have larger internal cavity size (7.6, 11.7A°, respectively) than 2-hydroxy propyl-β-cyclodextrin (5.7A°).[65] 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-cyclodextrin and calix-6,8-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.[55] Table 1 shows the results obtained over a period of two months for different assemblies of each sensor.

Typical calibration plots are shown in Fig. 4. The slopes of the calibration plots are 50.4, 54.3, 56.3 and 59.1 mV/concentration decade for sensors 1, 2, 3 and 4, 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. [47] 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 28, 35, 42 and 49 days for sensors 1, 2, 3 and 4, respectively. Sensors 3 and 4 display more stability and higher lifetime than sensors 1 and 2. This can be attributed to the preferential interaction between the PAM cation and very polar sulphonic acid groups present in the larger internal cavity size structure calix[6,8]arene.[66]

Table 1: Electrochemical response characteristics of the four investigated PAM sensors.

Parameter	Sensor (1)	Sensor (2)	Sensor (3)	Sensor (4)
Slope (mV/decade)[a]	50.4	54.3	56.3	59.1
Intercept (mV)[a]	147.6	211.0	319.2	281.8
Correlation coefficient (r)	1.0	1.0	1.0	1.0
Concentration range (mol L^{-1})	1.0×10^{-4}–1×10^{-2}	1.0×10^{-5}–1×10^{-2}	1.0×10^{-6}–1×10^{-2}	1.0×10^{-6}–1×10^{-2}
Response time (s)	45	35	20	15
Working pH range	3–6	3–6	3–6	3–6
LOD (mol L^{-1})[b]	3.6×10^{-5}	4.8×10^{-6}	6.8×10^{-7}	4.2×10^{-7}
Stability (days)	28.0	35.0	42.0	49.0
Average accuracy (%)	99.8	99.6	99.9	99.7
Standard deviation (precision)	1.0	0.5	0.8	0.5
Relative standard deviation (precision %)	1.0	0.5	0.8	0.5
Repeatability[c] (%)	0.7	0.5	0.8	0.5
Reproducibility[d] (%)	0.4	0.8	0.6	0.6

[a] Average of five determinations. [b] Limit of detection (measured by interception of the extrapolated arms of Fig. 4). [c] n The intraday (n = 3) and [d] n the interday (n = 3) relative standard deviations of samples concentrations (1×10^{-4}, 1×10^{-3}, and 1×10^{-2} mol L^{-1}) of PAM.

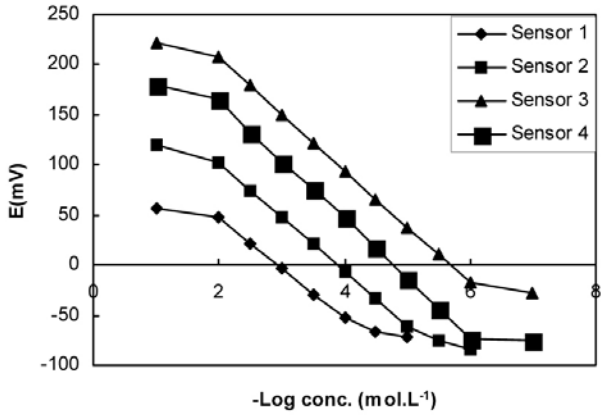

Fig. 4. Profile of the potential in mV versus–log concentrations of PAM in mol L^{-1} obtained with sensors 1, 2, 3 and 4.

The detection limits of the four sensors were estimated according to the IUPAC definition.[55] Table 1 shows that sensors 3 and 4 can detect PAM in very dilute solutions down 6.8×10^{-7} and 4.2×10^{-7} mol L^{-1} respectively. This agrees with the idea that PAM is typically bonded with the very polar sulphonic acid groups. The results obtained for the determination of PAM in pure powder form were compared with those obtained by using an official method.[2] No significant difference in results was found (Table 2).

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 PAM concentration by up to 10-fold. The required time for the sensors to reach values within ±1 mV of the final equilibrium potential was 45, 35, 20 and 15 s for sensors 1, 2, 3 and 4, respectively (Table 1).

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 four sensors are fairly constant over the pH range 3.0–6.0. Above pH 6.0 the potentials show sharp decrease due to the formation of non protonated amino group of PAM. While below pH 3.0, the response of electrodes decrease with the increase of analyte acidity; as at such high acidity the dissociation of the OH^- groups of 2HP β-CD and sulphonic acid groups of calix[6,8]arene is limited, also the membrane may extract H^+, furthermore, the neutralization of OH^- groups of 2HP β-CD and sulphonic acid groups of calix[6,8]arene takes place and the inclusion function of the ionospheres is decreased leading to weak responses.[47] Therefore the pH range from 3.0 to 6.0 was assumed to be the working pH range of the four sensors and so the drug was dissolved in acetate buffer pH 5.0 (Figs. 5), (Table 1). 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 3 shows the potentiometric selectivity coefficients of the proposed sensors in the presence of hydrocortisone acetate, and some 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, 3 and 4 are at least 10–100 times more selective than sensor 1. Sensors 3 and 4 display higher selec-

Table 2: Statistical analysis between the results obtained for the determination of PAM in pure sample by the proposed sensors and those obtained by the official method[2]

Item	Sensor 1	Sensor 2	Sensor 3	Sensor 4	Official method[USP2011 a]
Mean	99.8	99.6	99.9	99.7	100.1
S.D.	1.0	0.5	0.8	0.5	0.6
RSD%	1.0	0.5	0.8	0.5	0.6
Variance	1.0	0.3	0.6	0.3	0.4
n	5	5	5	5	5
F test (6.39)[b]	2.5	1.3	1.5	1.3	
Student's t test (2.306)[b]	0.6	1.2	0.7	1.1	

[a] HPLC method using acetonitrile: phosphate buffer (pH 7.5) as a mobile phase (55:45), UV detection at 224 nm and a flow rate of 2 ml/min. [b] Figures between parenthesis are the corresponding tabulated values (P = 0.05)

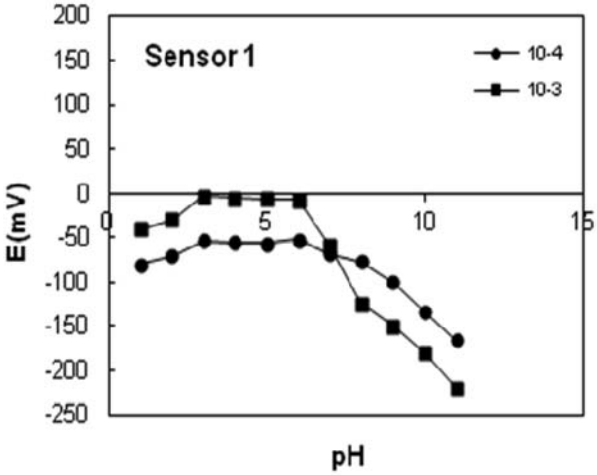

Fig 5a: Effect of pH on the response of the sensor 1 (1.0×10^{-3} – 1.0×10^{-4} mol L^{-1})

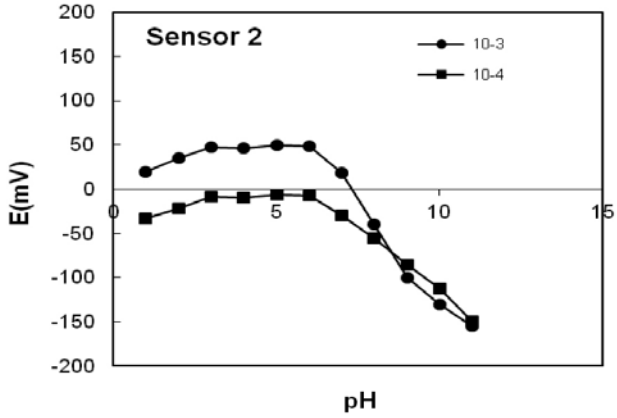

Fig 5b: Effect of pH on the response of the sensor 2 (1.0×10^{-3} – 1.0×10^{-4} mol L^{-1})

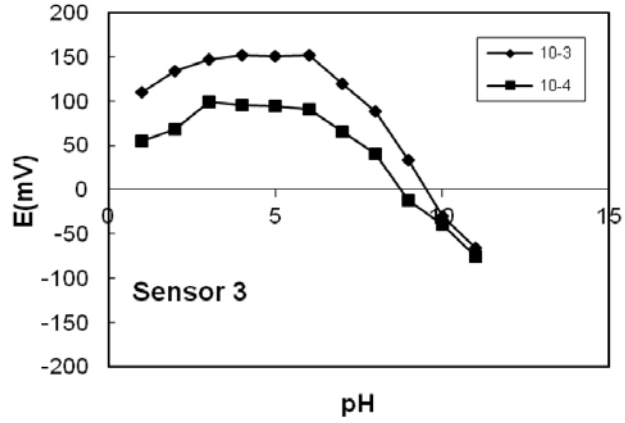

Fig 5c: Effect of pH on the response of the sensor 3 (1.0×10^{-3} – 1.0×10^{-4} mol L^{-1}).

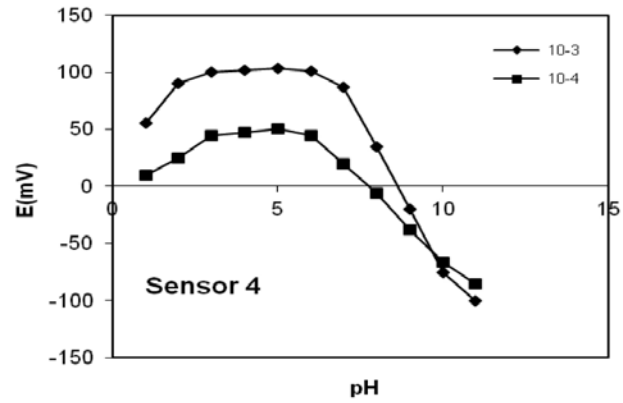

Fig 5d: Effect of pH on the response of the sensor4 (1.0×10^{-3} – 1.0×10^{-4} mol L^{-1}).

tivity and lower response for the potentially interfering species and co-formulated drug (hydrocortisone acetate) than sensor 2. This can be attributed to the preferential interaction between the PAM cation and very polar sulphonic acid groups present in the calix[6,8]arene structure.[66]

3. 5. Potentiometric Determination of PAM in Pharmaceutical Formulation

The proposed sensors were applied for the analysis of PAM pharmaceutical formulation. The results prove the applicability of the four sensors for the determination

Table 3: Potentiometric selectivity coefficients (average of five determinations) ($-log\ K^{Pot}_{PAM,\ I}$) of the proposed sensors by separate selectivity method.

Interferent[a]	Sensor 1	Sensor 2	Sensor 3	Sensor 4
Urea	3.0×10^{-2}	6.5×10^{-3}	2.0×10^{-3}	9.4×10^{-4}
Glycine	2.5×10^{-2}	8.1×10^{-3}	3.8×10^{-3}	4.1×10^{-3}
NH_4CL	3.7×10^{-1}	2.9×10^{-2}	6.0×10^{-4}	5.3×10^{-3}
KCl	4.8×10^{-1}	6.4×10^{-3}	1.8×10^{-4}	6.9×10^{-4}
NaCl	5.9×10^{-2}	3.7×10^{-4}	5.6×10^{-3}	2.8×10^{-4}
$CaCl_2$	3.9×10^{-1}	5.9×10^{-3}	8.7×10^{-3}	5.9×10^{-3}
$MgCl_2$	7.7×10^{-1}	4.4×10^{-2}	6.1×10^{-3}	4.1×10^{-3}
Hydroxyl amine	7.1×10^{-2}	4.8×10^{-3}	9.2×10^{-4}	1.1×10^{-4}
Hydrocortisone acetate	8.6×10^{-1}	9.1×10^{-2}	2.7×10^{-3}	2.3×10^{-4}

[a] All interferents were in the form of 1.0×10^{-3} mol L^{-1} solutions

Table 4: Determination PAM in pharmaceutical formulation by the proposed potentiometric method

Pramacort cream	Recovery % ± RSD %[a]			
B.N. 92308	Sensor 1	Sensor 2	Sensor 3	Sensor 4
1.0×10^{-3} mol L^{-1}	99.2 ± 0.7	98.6 ± 0.5	101.0 ± 0.2	98.7 ± 0.8
1.0×10^{-4} mol L^{-1}	99.6 ±0.4	100.1 ± 0.4	99.6 ± 0.2	99.3 ± 0.3

[a] Average of five determinations

of pharmaceutical formulation containing PAM. However, results show that hydrocortisone acetate does not interfere with PAM measurements using the four proposed sensors. These data are shown in Table 4.

3. 6. Potentiometric Determination of PAM in Plasma

The results obtained for the determination of PAM 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, 3 and 4 are more sensitive than sensor 1 in plasma samples. It is concluded that the proposed sensors can be successfully applied to in vitro studies and for clinical use.

Table 5: Determination of PAM in spiked human plasma by the proposed potentiometric method

Concentration	Recovery % ± RSD %[a]			
mol L^{-1}	Sensor 1	Sensor 2	Sensor 3	Sensor 4
1.0×10^{-3}	98.3 ± 0.1	99.9 ± 0.4	100.4 ± 0.7	98.4 ± 0.5
1.0×10^{-4}	99.4 ± 0.6	100.2 ± 0.2	101.2 ± 1.1	99.7 ± 1.0
1.0×10^{-5}	–	101.2 ± 0.7	99.6 ± 0.8	100.5 ± 0.6

[a] Average of three determinations.

3. 7. Validation

Linearity was assessed by the determination of the same concentration range as the calibration graphs using the four proposed sensors. The mean accuracies are given in Table 1. To evaluate precision and accuracy of the proposed sensors, three concentrations of PAM within the linear range (1×10^{-4}, 1×10^{-3}, and 1×10^{-2} M) were chosen. Three solutions of each concentration were prepared and analyzed in triplicate (repeatability assay). This assay was repeated on three different days (reproducibility assay). Table 1 shows all validation parameters of the proposed method including linearity, range, accuracy and precision.

4. Conclusion

The described sensors are sufficiently simple and selective for the quantitative determination of PAM in pure form, pharmaceutical formulation and in plasma. The use of 2-hydroxy propyl-β-cyclodextrin and sulphonated calix-6,8-arene as ionophores increased the membrane sensitivity and selectivity as in sensors 2, 3 and 4 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 PAM in quality-control laboratories. The proposed method has distinct advantage regarding sensitivity with other reported analytical methods. The proposed method is definitely superior to other reported methods.

5. References

1. *Martindale, The Complete Drug Reference* Pharmaceutical Press, London, UK, **2009**.
2. *USP 34-NF 29 US Pharmacopeia National Formulary*, Twinbrook Parkway, Rockville, **2011**.
3. R. Weinberger, R. Mann, J. Posluszny, *J. Pharm. Sci.*, **1980**, *69,* 475–477.
4. Z. L. Chang, J. P. Boller, D. M. pacenti, C. F. Wong, *J. Chromatogr. A*, **1984**, 301, 428–433.
5. R. Gill, R. W. Abbott, A. C. Moffat, *J. Chromatogr.*, **1984**, *301*, 155–163.
6. I. Jane, A. Mckinnon, R. J. Flanagan, *J. Chromatogr.*, **1985**, *323(2),* 191–225.
7. W. Li, L. Hua, *Journal of Northwest University*, **2007**, *1,* 52–54.
8. P. Tavlarakis, J. Greyling, N. H. Snow, *Anal. Methods*, **2010**, *2,* 722–727.
9. A. C. Moffat, K. W. Smalldon, *J. Chromatogr.*, **1974**, *90, 1, 13,* 9–17.
10. R. N. Goyal, V. K. Gupta, M. Oyama, N. Bachheti, *Electrochem. Commuin.*, **2005**, *7(8),* 803–807.
11. R. N. Goyal, V. K. Gupta, A. Sangal, N. Bachheti, *Electroanalysis*, **2005**, *17(24),* 2217–2223.

12. R. N. Goyal, V. K. Gupta, M. Oyama,N. Bachheti, *Electrochem. Commuin.*, **2006**, *8(1),* 65–70.
13. R. N. Goyal, V. K. Gupta, M. Oyama, N. Bachheti, *Talanta*, **2007**, *71(3),* 1110–1117.
14. R. N. Goyal, V. K. Gupta, M. Oyama, N. Bachheti, *Talanta*, **2007**, *72(3),* 976–983.
15. R. N. Goyal, V. K. Gupta, N. Bachheti, *Anal. Chim. Acta*, **2007**, *597(1),* 82–89.
16. R. N. Goyal, V. K. Gupta, S. Chatterjee, *Electrochimica Acta*, **2008**, *53(16),* 5354–5360.
17. R. N. Goyal, M. Oyama, V. K. Gupta, S. P. Singh, R. A. Sharma, *Sens. Actuators, B: Chemical*, **2008**, *134(2),* 816–821.
18. R. N. Goyal, V. K. Gupta, N. Bachheti, R. A. Sharma, *Electroanalysis*, **2008**, *20(7),* 757–764.
19. R. N. Goyal, V. K. Gupta, S. Chatterjee, *Talanta*, **2008**, *76(3),* 662–668.
20. R. N. Goyal, V. K. Gupta, S. Chatterjee, *Biosens. Bioelectron.*, **2009**, *24(6),* 1649–1654.
21. R. N. Goyal, V. K. Gupta, S. Chatterjee, *Biosens. Bioelectron.*, **2009**, *24(12),* 3562–3568.
22. R. Jain, V. K. Gupta, N. Jadon, K. Radhapyari, *J. Electroanal. Chem.*, **2010**, *648(1),* 20–27.
23. V. K. Gupta, R. Jain, N. Jadon, K. Radhapyari, *Journal of Colloid Interface Sci.*, **2010**, *350(1),* 330–335.
24. R. Jain, V. K. Gupta, N. Jadon, K. Radhapyari, *Anal. Biochem.*, **2010**, *407(1),* 79–88.
25. V. K. Gupta, R. Jain, S. Agarwal, R. Mishra, A. Dwivedi, *Anal. Biochem.*, **2011**, *410(2),* 266–271.
26. V. K. Gupta, R. Jain, K. Radhapyari, N. Jadon, S. Agarwal, *Anal. Biochem.*, **2011**, *408(2),* 179–196.
27. S. K. Srivastava, V. K. Gupta, M. K. Dwivedi, S. Jain, *Anal. Proc. Incl. Anal. Commun.*, **1995**, *32(1),* 21–23.
28. A. K. Jain, V. K. Gupta, L. P. Singh, *Anal. Proc. Incl. Anal. Commun.*, **1995**, *32(7),* 263–266.
29. A. K. Jain, V. K. Gupta, B. B. Sahoo, L. P. Singh, *Anal. Proc. Incl. Anal. Commun.*, **1995**, *32(3),* 99–101.
30. S. K. Srivastava, V. K. Gupta, S. Jain, *Electroanalysis*, **1996**, *8(10),* 938–940.
31. V. K. Gupta, A. K. Jain, L. P. Singh, U. Khurana, *Anal. Chim. Acta*, **1997**, *355(1),* 33–41.
32. A. K. Jain, V. K. Gupta, U. Khurana, L. P. Singh, *Electroanalysis*, **1997**, *9(11),* 857–860.
33. V. K. Gupta,P. Kumar, *Anal. Chim. Acta*, **1999**, *389(1–3),* 205–212.
34. R. Prasad, V. K. Gupta, A. Kumar, *Anal. Chim. Acta*, **2004**, *508(1),* 61–70.
35. V. K. Gupta, A. K. Singh, B. Gupta, *Anal. Chim. Acta*, **2006**, *575(2),* 198–204.
36. V. K. Gupta, A. K. Jain, P. Kumar, S. Agarwal, G. Maheshwari, *Sens. Actuators, B: Chemical*, **2006**, *113(1),* 182–186.
37. A. K. Singh, V. K. Gupta, B. Gupta, *Anal. Chim. Acta*, **2007**, *585(1),* 171–178.
38. V. K. Gupta, R. N. Goyal, R. A. Sharma, *Int. J. Electrochem. Sci.*, **2009**, *4,* 156–172.
39. V. K. Gupta, M. Al Khayat, A. K. Singh, M. K. Pal, *Anal. Chim. Acta*, **2009**, *634(1),* 36–43.
40. V. K. Gupta, R. N. Goyal, R. A. Sharma, *Anal. Chim. Acta*, **2009**, *647(1),* 66–71.
41. V. K. Gupta, M. R. Ganjali, P. Norouzi, H. Khani, A. Nayak, S. Agarwal, *Crit. Rev. Anal. Chem.*, **2011,** *41(4),* 282–313.
42. K. Birgit, F. Christopher, S. Roswitha, B. Georg, W. Udo, S. Volker,G. G nter, *Anal. Chem.*, **2002**, *74(13),* 3005–3012.
43. R. I. S. Staden, R. M. Nejem, *Sens. Actuators, B: Chem.*, **2006**, *117(1),* 123–127.
44. S. R. Patil, M. Turmine, V. Peyre, G. Durand, B. Pucci, *Talanta*, **2007**, *74(1)*, 72–77.
45. K. I. Ozoemena, R. I. Stefan, *Talanta*, **2005**, *66(2),* 501–504.
46. A. M. El-Kosasy, *J. AOAC Int.*, **2003**, *86(1),* 15–21.
47. A. M. EL-Kosasy, M. Y. Salem, M. G. EL-Bardicy, M. K. Abdelrahman, *J. AOAC Int.*, **2009**, *92(6),* 1631–1638.
48. V. K. Gupta, R. Mangla, S. Agarwal, *Electroanalysis*, **2002**, *14(15–16),* 1127–1132.
49. V. K. Gupta, R. Ludwig, S. Agarwal, *Anal. Chim. Acta*, **2005**, *538(1–2),* 213–218.
50. A. K. Jain, V. K. Gupta, L. P. Singh, P. Srivastava, J. R. Raisoni, *Talanta*, **2005**, *65(3),* 716–721.
51. F. Kivlehan, W. J. Mace, H. A. Moynihan, D. W. M. Arrigan, *Anal. Chim. Acta.*, **2007**, *585(1),* 154–160.
52. P. Kumar,Y.B. Shim, *Talanta*, **2009**, 77(3), 1057–1062.
53. S. J. Park, O. J. Shon, J. A. Rim, J. K. Lee, J. S. Kim, H. Nam, H. Kim, *Talanta*, **2001**, *55(2).*
54. *Vogel's Text Book of Quantitative Inorganic Analysis Including Elementary Instrumental Analysis*, Published for ELBS, **1978**.
55. IUPAC, **2000**, *72,* 1851.
56. S. K. Mittal, A. Kumar, N. Gupta, S. Kaur, S. Kuma, *Anal. Chim. Acta*, **2007**, *585(1),* 161–170.
57. A. R. Zanganeh,M. K. Amini, *Sens. Actuators B: Chem.*, **2008**, *135(1),* 358–365.
58. L. Gorski, A. Matusevich, P. Parzuchowski, I. uciuk, E. Malinowska, *Anal. Chim. Acta*, **2010**, *665(1),* 39–46.
59. E. E. Sideris, G. N. Valsami, M. A. Koupparis, P. E. Macheras, *Eur. J. Pharm. Sci.*, **1999**, *7(4),* 271–278.
60. S. Flink, F. C. J. M. van Veggel, D. N. Reinhoudt, *Adv. Mater.*, **2000**, *12(18),* 1315–1328.
61. E. Bakker,Y. Qin, *Anal. Chem.*, **2006**, *78(12),* 3965–3984.
62. L. Chen, J. Zhang, W. Zhao, X. He, Y. Liu, *J. Electroanal. Chem.*, **2006**, *589(1),* 106–111.
63. M. Zareh, B. Malinowska, *J. AOAC Int.*, **2007**, *90(1),* 147–152.
64. A. M. El-Kosasy, M. Nebsen, M. K. Abd El-Rahman, M. Y. Salem, M. G. El-Bardicy, *Talanta*, **2011**, *85,* 913–918.
65. K. Hamilton, *SYNTHESIS, CHARACTERIZATION, AND APPLICATION OF WATER-SOLUBLE CHIRAL CALIX [4] ARENE DERIVATIVES IN SPECTROSCOPY AND CAPILLARY ELECTROKINETIC CHROMATOGRAPHY, 30*, **2003**.
66. S. Shinkai, A. Ikeda, *Pure & Appl. Chem*, **1999,** *71(2),* 275–280.

Povzetek

Preizkusili smo štiri nove elektrode, selektivne za pramoksin hidroklorid (PAM), narejene z 2-nitrofeniloktil etrom kot plastifikatorjem v polimerni matrici iz polivinilklorida (PVC). Senzor 1 je bil izdelan z natrijevim tetrafenilboratom (TPB) kot anionskim izmenjevalcem brez vključitve ionoforja. Pri senzorju 2 smo kot ionofor uporabili 2-hidroksipropilciklodekstrin, pri senzorju 3 pa 4-sulfokaliks-6-aren in 4-sulfokaliks-8-aren pri senzorju 4. Linearen odziv za PAM je bil v koncentracijskem območju $1{,}0 \times 10^{-4} - 1{,}0 \times 10^{-2}$ mol L^{-1} pri senzorju 1, $1{,}0 \times 10^{-5} - 1{,}0 \times 10^{-2}$ mol L^{-1} pri senzorju 2 ter $1{,}0 \times 10–6 - 1{,}0 \times 10^{-2}$ mol L^{-1} pri senzorjih 3 in 4. Znotraj pH območja 3,0–6,0 smo opazili Nernstov naklon 50,4 ± 0,6, 54,3 ± 0,8, 56,3 ± 0,3 in 59,1 ± 0,5 mV/dekado. Selektivnostni koeficienti za razvite senzorje so kazali na odlično selektivnost za PAM. Uporaba 2-hidroksipropilciklodekstrina (2HP-β-CD) ter 4-sulfokaliks [6, 8] arena (SC 6, 8) kot ionoforjev je znatno vplivala na zvišanje občutljivosti in selektivnosti membrane pri senzorjih 2, 3 in 4 v primerjavi s senzorjem 1. Predlagani senzorji so imeli ugodne analizne karakteristike za določitev PAM v praškovnem proizvodu, farmacevtski obliki in v bioloških tekočinah. Z validacijo metode smo pokazali ustreznost predlaganih elektrod za uporabo pri kontroli kvalitete učinkovine. Izvedli smo tudi statistično primerjavo med rezultati, dobljenimi s predlagano metodo, in rezultati uradne metode za učinkovino. Med njimi ni bilo signifikantnih razlik.