

Novel PVC Membrane Sensors for Pinaverium Bromide Determination in Pharmaceutical Formulation and Human Urine

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Abstract: The characteristics, performance and applications of three new, simple, rapid, selective and sensitive poly vinyl chloride (PVC) membrane electrodes are described for determination of pinaverium bromide (Pina) in raw material, pharmaceutical formulations and human urine using dibutylphthalate (DBP) as plasticizer. Sensor no. 5 (Pina-TPB) was fabricated using cation exchanger sodium tetraphenylborate (NaTPB), sensor no. 7 (Pina- RT) used ammonium reineckate (RT) and sensor no. 13 (Pina-TPB/RT) used a mixture of both. The sensors showed a near-Nernstian slope of 58.7 ± 0.26 , 54.4 ± 0.34 and 55.4 ± 0.27 mV decade⁻¹ at $25 \pm 0.1^\circ\text{C}$ within the concentration range 5×10^{-6} - 5×10^{-3} , 9.8×10^{-6} - 5×10^{-3} and 9.8×10^{-6} - 5×10^{-3} mol L⁻¹ Pina, with detection limits of 2×10^{-6} , 5×10^{-6} and 5×10^{-6} mol L⁻¹ Pina for sensors, respectively. The electrode response is independent of pH in the range of 3-11 for the three sensors investigated. All sensors show fast response time which is very short (10 s). Surface analysis using scanning electron microscopy was used to determine the cause of the limited life span of plastic membrane electrode. Validation of the method showed suitability of the sensors for the quality control analysis of pinaverium bromide in pharmaceutical formulation and human urine. The proposed method was simple, accurate and precise.

Keywords: Pinaverium bromide; Potentiometry; Sensors; Electron microscopy Pharmaceutical formulations; Human urine.

1 Introduction

Pinaverium bromide (Fig. 1) [Morpholinium, 4-[(2-bromo-4,5-dimethoxyphenyl)methyl]-4-[2-[2-(6,6-dimethylbicyclo[3.1.1]hept-2-yl)ethoxy]ethyl]-, bromide (Mol. Wt. 591.42)] is a quaternary ammonium compound, is an L-type calcium channel blocker with selectivity for the gastrointestinal tract, which can effectively relieve pain, diarrhea and intestinal discomfort, and provide safe and effective treatment of irritable bowel syndrome (IBS)^[1-4].

Several methods have been reported for the determination of pinaverium bromide in dosage forms and in biological fluids: liquid chromatography–electrospray tandem mass spectrometry^[5], high-performance liquid chromatography^[6-7], gas chromatographic mass spectrometry^[8], but these methods suffer from a variety of drawbacks, i.e. they are costly, and are not appropriate for large-scale monitoring.

Potentiometric sensors can overcome these limitations and have advantages such as selectivity, sensitivity, high precision, simplicity, wide linear range, low cost, and rapid determination of a variety of ions in different types of sample. Pinaverium has a low absorption (8–10%), and maximum blood levels are reached 1 h after oral administration. Some 97% of the drug is bounded to protein in the plasma. With 1.5 h mean half-life, it undergoes a first-pass metabolism that reduces the bioavailability at therapeutic doses. Pinaverium bromide is almost eliminated after transformation in the liver^[9]. One of the major problems in the measurement of pinaverium bromide is the small amount present. Thus, there is a growing need for robust sensor systems that allow rapid and reliable determinations, particularly in quality control analysis.

No studies in the literature have reported potentiometric methods for determination of pinaverium bromide yet.

The present work describes construction and investigation of performance characteristics of new ISEs based on PVC for the determination of pinaverium bromide in bulk powder, pharmaceutical formulations, and spiked urine.

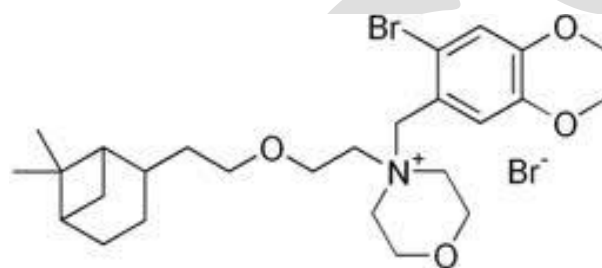


Fig. 1. Chemical structure of pinaverium bromide

2 Experimental

2.1 Reagents and materials

All chemicals were of analytical grade. Double distilled water was used throughout all experiments. Pure grade pinaverium bromide and the pharmaceutical preparation spascolon tablets (100 mg/Tablet) were provided by Chemipharm pharmaceutical industries, 6th October City- Egypt. Sodium tetraphenylborate (NaTPB), ammonium reineckate (NH₄RT), poly (vinyl chloride) of high molecular weight (PVC), dioctyl sebacate (DOS), and tricresyl phosphate (TCP) were obtained from Fluka (U.S.A.). Tetrahydrofuran (THF), dibutyl phthalate (DBP) and dioctyl phthalate (DOP) were purchased from Merck (Germany). The metal salts were provided by BDH as nitrates or chlorides. Stock solutions of the metal salts were prepared in bidistilled water and standardized when-ever necessary.

2.2 Apparatus

Potentiometric and pH-measurements were carried out using 702 titroprocessor equipped with a 665 dosimat (Switzerland) made by Metrohm. A mLw W20 circulator thermostat was used to control the temperature of the test solutions. A saturated calomel electrode (SCE) was used as the external reference, while a Ag/AgCl electrode was used as an internal reference. The electrochemical system may be represented as follows: Ag/AgCl/filling solution/membrane/test solution//KCl salt bridge//saturated calomel electrode.

JEOL scanning electron microscope (JSM - 5610 LA) is used to investigate the morphology of the surfaces of freshly prepared and expired electrode membranes.

2.3 Preparation of the ion-pair

The ion-pairs, Pina-TPB and Pina-RT were prepared by mixing 100 mL 10^{-2} mol L⁻¹ pinaverium bromide solutions with 100 mL of 10^{-2} mol L⁻¹ of sodium tetraphenylborate or ammonium reineckate. The formed precipitates were filtered, washed thoroughly with bidistilled water and dried at room temperature. The composition of the ion-pair was found to be 1: 1 both in case of Pina-TPB and Pina-RT as confirmed by elemental analysis data. The percentage values found are 71.89, 7.38 and 1.86 and the calculated values are 72.22, 7.34 and 1.68 for C, H and N, respectively, in case of Pina-TPB, while in case of Pina-RT the percentage values found are 42.32, 5.49 and 11.46 and the calculated values are 42.35, 5.52 and 11.53 for C, H and N, respectively.

2.4 Conductometric measurements

Conductometric titrations were followed with a Jenway conductivity meter. 5 mL of 5.0×10^{-3} mol L⁻¹ pinaverium bromide solution diluted to 50 mL with distilled water was titrated against a 1.0×10^{-2} mol L⁻¹ NaTPB or NH₄RT solution. The conductance of the solution was measured after each addition of the titrant. The titration plot (conductance versus molar ratio) showed a break which corresponds to stoichiometry of ion-pairs.

2.5 Electrode preparation

The electrode was constructed as described previously^[10]. The membranes were prepared by dissolving varying amounts of the ion-pair and PVC in 10 mL THF. To these, solvent mediators, viz. DBP, DOS, TCP and DOP were added to get membranes of different compositions. The mixture was stirred with a glass rod. When the solution became viscous it was poured into a 6.0 cm Petri dish. The solution was then allowed to evaporate for 24 h at room temperature. Transparent membranes of about 0.2 mm thickness were obtained. A 12 mm diameter disk was cut out from the prepared membrane and glued using PVC-THF paste to the polished end of a plastic cap attached to a glass tube. The electrode body was filled with a solution of 1×10^{-1} mol L⁻¹ NaCl and 5×10^{-3} mol L⁻¹ pinaverium bromide solution. The electrode was preconditioned before use by soaking in a 1×10^{-3} mol L⁻¹ pinaverium bromide solution for 0.5 h. The ratio of membrane ingredients, time of contact and concentration of conditioning solution were optimized so that the potentials recorded were reproducible and stable.

2.6 Electrodes calibration

The conditioned electrodes were calibrated by separately 50 mL aliquots of solutions (10^{-6} to 5×10^{-3} mol L⁻¹) of pinaverium bromide into a series of 100 mL beakers. The membrane electrodes, in conjunction with saturated calomel electrode, were immersed in above test solutions and allowed to equilibrate while stirring. The potential was recorded after stabilising to ± 1 mV, and the potential was plotted as a function of the negative logarithm of pinaverium bromide concentration.

2.7 Selectivity coefficient determination

The modified separate solution method and the matched potential method (MPM)^[11-13] are employed to determine the selectivity coefficients, $\log K_{\text{Pina}^J\text{Z}^+}^{\text{pot}}$, of the potentiometric sensors towards different species. In the modified separate solution method, the potential of a cell comprising a working electrode and a reference electrode is measured in two separate solutions, where, E_1 is the potential measured in 1×10^{-3} mol L⁻¹ Pina, E_2 the potential measured in 1×10^{-3} mol L⁻¹ of the interfering compound, z_1 and z_2 are the

charges of pinaverium and interfering species, respectively and S is slope of the electrode calibration plot. The selectivity coefficients were determined by the modified separate solution method using the rearranged Nicolsky equation:

$$\log K_{\text{Pina},J}^{\text{pot}} = ((E_1 - E_2)/S) + (1 + (z_1/z_2)) \log a$$

Potentiometric selectivity factors of the electrode were evaluated by applying the matched potential method (MPM). According to this method, the activity of Pina solution was increased from $a_A = 1.0 \times 10^{-6} \text{ mol L}^{-1}$ (reference solution) to $\hat{a}_A = 1 \times 10^{-3} \text{ mol L}^{-1}$, and the changes in potential (ΔE) corresponding to this increase were measured. Next, a solution of an interfering ion of concentration a_B in the range $1.0 \times 10^{-1} - 1.0 \times 10^{-2} \text{ mol L}^{-1}$ was added to new $1 \times 10^{-6} \text{ mol L}^{-1}$ reference Pina solution until the same potential change (ΔE) was recorded. The selectivity factor $K_{A,B}^{\text{MPM}}$ for each studied species was calculated using the following equation:

$$K_{A,B}^{\text{MPM}} = \frac{(\hat{a}_A - a_B)}{a_B}$$

2.8 Determination of pinaverium bromide in spascolon tablets

The required amount from the tablets was dissolved in 30 mL bidistilled water and filtered in 50 mL measuring flask. The residue was washed three times with bidistilled water, and the volume was completed to the mark by the same solvent. The contents of the measuring flask were transferred into a 100 mL titration cell and subjected to potentiometric determination of pinaverium bromide.

2.9 Determination of pinaverium bromide in spiked urine samples

Different amounts ($5 \times 10^{-5} - 2 \times 10^{-4} \text{ mol L}^{-1}$) of pinaverium bromide and 5 mL urine of a healthy person were transferred to 50-mL measuring flask and completed to the mark by bidistilled water. The contents of the measuring flask were transferred to a 100-mL beaker, and subjected to potentiometric determination of pinaverium bromide by the standard addition method.

3 Results and discussion

3.1 Influence of membrane composition

Pinaverium cation was found to form 1:1 water insoluble ion-pair with each of sodium tetraphenylborate and ammonium reineckate as indicated by elemental analysis data and ascertained using conductometric titration (Fig. 2). The prepared ion-pairs were identified and examined as ion exchange sites in PVC membrane sensors responsive for pinaverium cation.

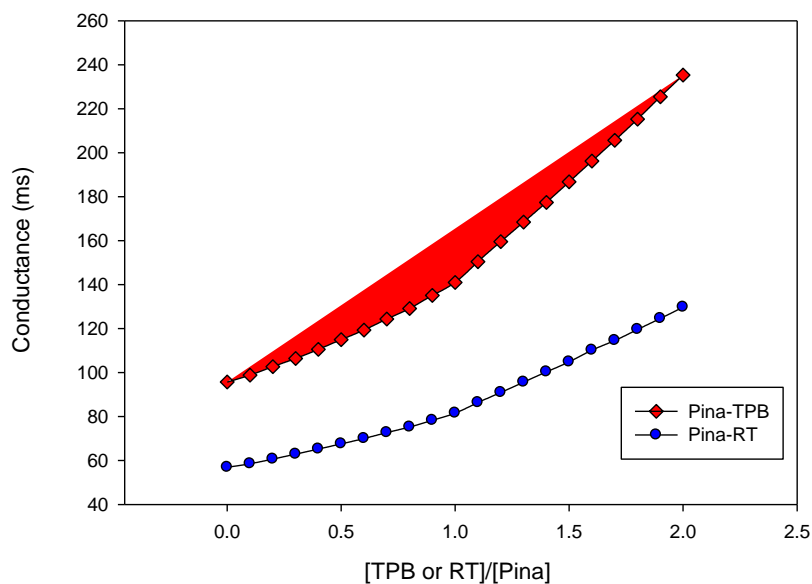


Fig. 2. Conductometric titration curves of $5 \times 10^{-3} \text{ mol L}^{-1}$ Pina against $1.0 \times 10^{-2} \text{ mol L}^{-1}$ NaTPB and RT

Several sensors of different compositions prepared as described in the experimental section, were tested. The slope, the linear concentration range, limit of detection and limit of quantitation for the different sensors are given in Table 1. The best performance was exhibited by sensor no. 5 with Pina-TPB, DBP and PVC in ratio 12:44:44, respectively. This sensor showed a nearly Nernstian response with slope of 57.6 mV/decade and a linear concentration range 5×10^{-6} - $5 \times 10^{-3} \text{ mol L}^{-1}$. Sensor no. 7 with Pina-RT, DBP and PVC in ratio 1:49.50:49.50, respectively showed a nearly Nernstian response with slope of 53.6 mV/decade and a linear concentration range 9.8×10^{-6} - $5 \times 10^{-3} \text{ mol L}^{-1}$ while sensor no. 13 with Pina-TPB/RT, DBP and PVC in ratio 2:1:48.50:48.50, respectively, showed a nearly Nernstian response with slope of 53.6 mV/decade and a linear concentration range 9.8×10^{-6} - $5 \times 10^{-3} \text{ mol L}^{-1}$. The calibration graphs of the different sensors are shown in Fig .3

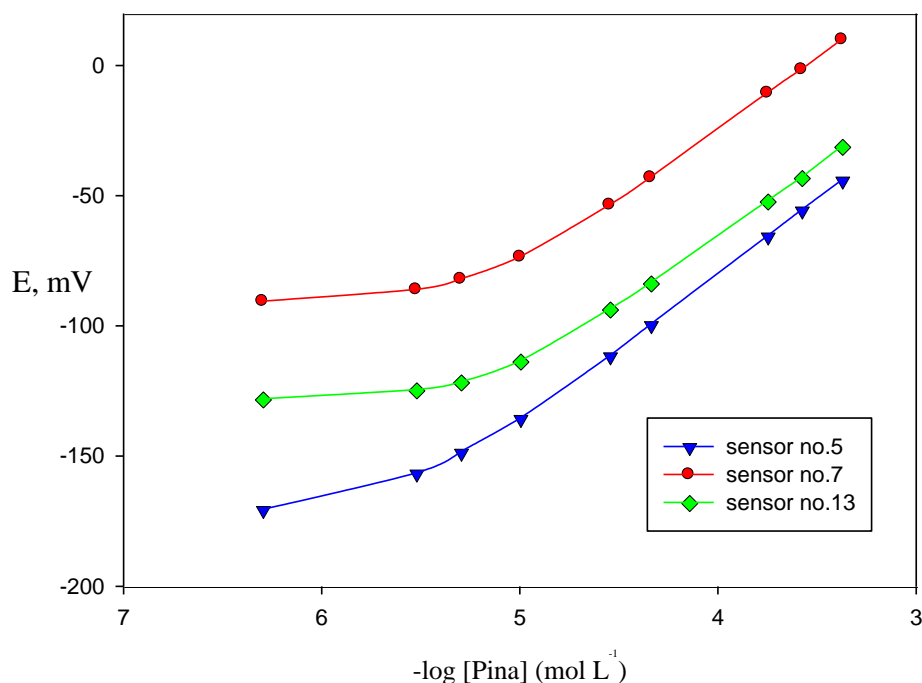


Fig. 3. Profile of the potential in mV versus $-\log$ concentrations of Pina in (mol L^{-1}) obtained with sensors nos. 5, 7 and 13

The PVC acts as a regular support matrix for the plastic membrane ion-selective electrode, but its use requires a plasticizer which acts as a fluidizer allowing homogeneous dissolution and diffusional mobility of the electroactive complex inside the membrane^[14]. Four plasticizers, DBP, DOP, DOS and TCP were tested and recorded in Table 1. The results indicated that DBP is the best tested plasticizer. Poor sensitivities for sensors plasticized by the other plasticizers are due to low distributions of the electroactive ion-pairs Pina-TPB and Pina-RT in these solvents. The electrochemical performance characteristics of the proposed sensors were systematically evaluated according to IUPAC standards^[15]. Table 2 shows that sensor no. 5 can detect Pina in very dilute solutions down to $2 \times 10^{-6} \text{ mol L}^{-1}$

Table 1. Optimization of membrane compositions and their potentiometric response for Pina ion selective membrane

Sensors no.	Composition of membrane% (w/w; mg)							Slope mV/decade	Linear concentration range (mol L ⁻¹)	LOD ^a (mol L ⁻¹)	LOQ ^b (mol L ⁻¹)	RSD %
	Pina-TPB	Pina-RT	PVC	DBP	DOP	DOS	TC P					
1	3	-	48.50	48.50	-	-	-	55.9±0.23	5x10⁻⁶-5x10⁻³	3x10⁻⁶	1x10⁻⁵	0.42
2	5	-	47.50	47.50	-	-	-	55.5±0.25	5x10⁻⁶-5x10⁻³	3x10⁻⁶	1x10⁻⁵	0.46
3	7	-	46.50	46.50	-	-	-	56.1±0.07	5x10⁻⁶-5x10⁻³	3x10⁻⁶	1x10⁻⁵	0.12
4	9	-	45.50	45.50	-	-	-	56.7±0.28	5x10⁻⁶-5x10⁻³	3x10⁻⁶	1x10⁻⁵	0.50
5	12	-	44	44	-	-	-	57.6±0.21	5x10⁻⁶-5x10⁻³	2x10⁻⁶	6.7x10⁻⁶	0.37
6	-	0.5	49.75	49.75	-	-	-	53.4±0.11	9.8x10⁻⁶-5x10⁻³	5x10⁻⁶	1.6x10⁻⁵	0.22
7	-	1	49.50	49.50	-	-	-	53.6±0.37	9.8x10⁻⁶-5x10⁻³	5x10⁻⁶	1.6x10 ⁻⁵	0.69
8	-	1.5	49.25	49.25	-	-	-	52.6±0.29	9.8x10⁻⁶-5x10⁻³	9.8x10⁻⁶	3.2x10⁻⁵	0.56
9	-	2	49.00	49.00	-	-	-	52.5±0.34	1.9x10⁻⁵-5x10⁻³	9.8x10⁻⁶	3.2x10⁻⁵	0.66
10	-	3	48.50	48.50	-	-	-	51.0±0.45	1.9x10⁻⁵-5x10⁻³	9.8x10⁻⁶	3.2x10⁻⁵	0.89
11	-	5	47.50	47.50	-	-	-	48.9±0.39	2.8x10⁻⁵-5x10⁻³	1.9x10 ⁻⁵	6.3x10 ⁻⁵	0.81
12	1	1	49.00	49.00	-	-	-	53.2±0.18	9.8x10 ⁻⁶ -5x10 ⁻³	4x10 ⁻⁶	1.3x10 ⁻⁵	0.34
13	2	1	48.50	48.50	-	-	-	53.6±0.39	9.8x10⁻⁶-5x10⁻³	5x10 ⁻⁶	1.6x10 ⁻⁵	0.73
14	12	-	44	-	44	-	-	48.7±0.36	5x10⁻⁶-5x10⁻³	1.4x10 ⁻⁶	4.7x10 ⁻⁶	0.86
15	12	-	44	-	-	44	-	49.9±0.34	5x10 ⁻⁶ -5x10 ⁻³	3x10 ⁻⁶	1x10 ⁻⁵	0.75
16	12	-	44	-	-	-	44	51.2±0.27	5x10 ⁻⁶ -5x10 ⁻³	2x10 ⁻⁶	6.7x10 ⁻⁶	0.55
17	-	1	49.50	-	49.50	-	-	36.8±0.41	9.8x10⁻⁶-5x10⁻³	5x10 ⁻⁶	1.6x10 ⁻⁵	0.88
18	-	1	49.50	-	-	49.50	-	47.5±0.28	9.8x10 ⁻⁶ -5x10 ⁻³	5x10 ⁻⁶	1.6x10 ⁻⁵	0.64
19	-	1	49.50	-	-	-	49.50	48.1±0.21	9.8x10⁻⁶-5x10⁻³	4x10 ⁻⁶	1.3x10 ⁻⁵	0.45

a Limit of detection

b Limit of Quantitation

RSD: relative standard deviation (four determinations)

Table 2. Electrochemical response characteristics of the three investigated Pina sensors

Parameter	Sensor no.5	Sensor no.7	Sensor no.13
^a Slope (mV/decade)	57.6±0.21	53.6±0.37	53.6±0.39
) LOD (mol L ⁻¹)	2x10 ⁻⁶	5x10 ⁻⁶	5x10 ⁻⁶
LOQ (mol L ⁻¹)	6.6x10 ⁻⁶	16.7x10 ⁻⁶	16.7x10 ⁻⁶
Response time (s)	10	10	10
Working pH range	3-11	3-11	3-11
linear concentration range (mol L ⁻¹)	5x10 ⁻⁶ -5x10 ⁻³	9.8x10 ⁻⁶ -5x10 ⁻³	9.8x10 ⁻⁶ -5x10 ⁻³
Stability (days)	16	6	16
Average recovery(%)±S.D. ^a	99.76±0.213	98.89±0.372	99.92±0.396
Correlation coefficient (r ²)	0.9999	0.9994	0.9996

^a Average of four determinations.

3.2 Influence of internal filling solution

The sensors have been examined at various concentrations of internal reference pinaverium bromide solution in the range of (5x10⁻³ to 1x10⁻⁴ mol L⁻¹) and the potential response of the electrodes has been observed. It was found that the best results in terms of slope and linear concentration range have been obtained with internal solution of concentration 5x10⁻⁴ mol L⁻¹. Thus, 5x10⁻⁴ mol L⁻¹ concentration of the reference solution was quite appropriate for the smooth functioning of the proposed sensors. The results are shown in Table 3.

Table 3. Effect of internal filling solution on pina responsive sensors

Internal filling solution (mol L ⁻¹)	Slope mV/decade	Linear concentration range (mol L ⁻¹)
Sensor no.5		
5 x10 ⁻³	57.6±0.24	5x10 ⁻⁶ -5x10 ⁻³
1 x10 ⁻³	57.7±0.28	5x10 ⁻⁶ -5x10 ⁻³
5 x10⁻⁴	58.7±0.26	5x10⁻⁶ -5x10⁻³
1 x10 ⁻⁴	57.3±0.29	5x10 ⁻⁶ -5x10 ⁻³
Sensor no.7		
5 x10 ⁻³	53.6±0.36	9.8x10 ⁻⁶ -5x10 ⁻³
1 x10 ⁻³	52.0±0.19	9.8x10 ⁻⁶ -5x10 ⁻³
5 x10⁻⁴	54.4±0.34	9.8x10⁻⁶ -5x10⁻³
1 x10 ⁻⁴	53.4±0.29	9.8x10 ⁻⁶ -5x10 ⁻³
Sensor no.13		
5 x10 ⁻³	53.6±0.34	9.8x10 ⁻⁶ -5x10 ⁻³
1 x10 ⁻³	53.0±0.26	9.8x10 ⁻⁶ -5x10 ⁻³
5 x10⁻⁴	55.4±0.27	9.8x10⁻⁶ -5x10⁻³
1 x10 ⁻⁴	54.2±0.22	9.8x10 ⁻⁶ -5x10 ⁻³

3.3 Dynamic response time

Dynamic response time is an important factor for analytical applications of ion-selective electrodes. To measure the dynamic response time of the sensor the concentration of the test solution was changed in steps from (1x10⁻⁶ – 1x10⁻³ mol L⁻¹). The required time for the sensors to reach values within ±1 mV of the final equilibrium potential was (5-10 s) for sensors.

3.4 Influence of soaking time and life time

The life times of the sensors were determined by soaking in 1x10⁻³ mol L⁻¹ pinaverium bromide solution for interval ranging from 0.5 hr. to 16 days till the electrode lost its Nernstian behavior. This behavior may be attributed to the decomposition of the ion-pair and loss of other components in the membrane phase that was in contact with aqueous test solution containing drug ion. The response of the sensors has been measured by recording the calibration graph at 25 °C at different intervals. The results listed in Table 4 show the effect of soaking time on slope and concentration range. It is worthy to mention that the short life time of the investigated electrodes (less than one month for the best cases) may be attributed to the low lipophilicity of pina –ion-pairs^[16].

Table 4. Effect of soaking time on the potential response of the pina sensors

Soaking time	Slope(mV/decade)	Linear concentration range (mol L ⁻¹)
Sensor no.5		
0.5 hr	58.7±0.21	5x10 ⁻⁶ -5x10 ⁻³
6 hr	58.5±0.24	5x10 ⁻⁶ -5x10 ⁻³
1 day	56.9±0.31	5x10 ⁻⁶ -5x10 ⁻³
3 days	55.8±0.22	6.5x10 ⁻⁶ -5x10 ⁻³
6 days	55.3±0.27	6.8x10 ⁻⁶ -5x10 ⁻³
7 days	54.0±0.19	8x10 ⁻⁶ -5x10 ⁻³
11 days	52.2±0.25	9.5x10 ⁻⁶ -5x10 ⁻³
16 days	45.3±0.26	2x10 ⁻⁵ -5x10 ⁻³
Sensor no.7		
0.5 hr	54.4±0.27	9.8x10 ⁻⁶ -5x10 ⁻³
6 hr	54.2±0.34	9.8x10 ⁻⁶ -5x10 ⁻³
1 day	53.3±0.37	9.8x10 ⁻⁶ -5x10 ⁻³
2 days	52.6±0.28	1x10 ⁻⁵ -5x10 ⁻³
3 days	50.7±0.25	1.5x10 ⁻⁵ -5x10 ⁻³
5 days	50.3±0.39	1.8x10 ⁻⁵ -5x10 ⁻³
6 days	48.9±0.37	2.2x10 ⁻⁵ -5x10 ⁻³
Sensor no.13		
0.5 hr	55.4±0.28	9.8x10 ⁻⁶ -5x10 ⁻³
2 hr	56.9±0.26	9.8x10 ⁻⁶ -5x10 ⁻³
6 hr	55.9±0.24	9.8x10 ⁻⁶ -5x10 ⁻³
1 day	55.2±0.28	9.8x10 ⁻⁶ -5x10 ⁻³
2 days	55.8±0.27	9.8x10 ⁻⁶ -5x10 ⁻³
3 days	55.5±0.25	9.8x10 ⁻⁶ -5x10 ⁻³
5 days	54.9±0.29	1x10 ⁻⁵ -5x10 ⁻³
6 days	53.5±0.21	1x10 ⁻⁵ -5x10 ⁻³
8 days	53.3±0.23	1.2x10 ⁻⁵ -5x10 ⁻³
12 days	51.5±0.21	1.5x10 ⁻⁵ -5x10 ⁻³
14 days	50.5±0.31	1.7x10 ⁻⁵ -5x10 ⁻³
16 days	49.0±0.32	1.9x10 ⁻⁵ -5x10 ⁻³

3.5 Influence of soaking on the morphology of the membrane's surface

The electron microscopy images of surfaces of fresh and expired electrodes were obtained by tracing the secondary electrons emitted from the membrane surface. Drastic changes in the morphology of the surfaces which can be attributed to prolonged soaking of the electrodes have been observed. Images for a membrane including Pina-RT ion pair is taken as a representative (Fig. 4). These morphological changes are ascribed to some sort of solvent/gel layer interaction leading ultimately to shrinking of the polymeric network [Fig. 4b]. The harm of these surface changes is that they generate unequal strains at the deformed areas and consequently produce asymmetry potentials^[17]. These potentials interfere with the phase boundary equilibrium of pinaverium at the membrane surface, and negatively affect the performance of the electrode.

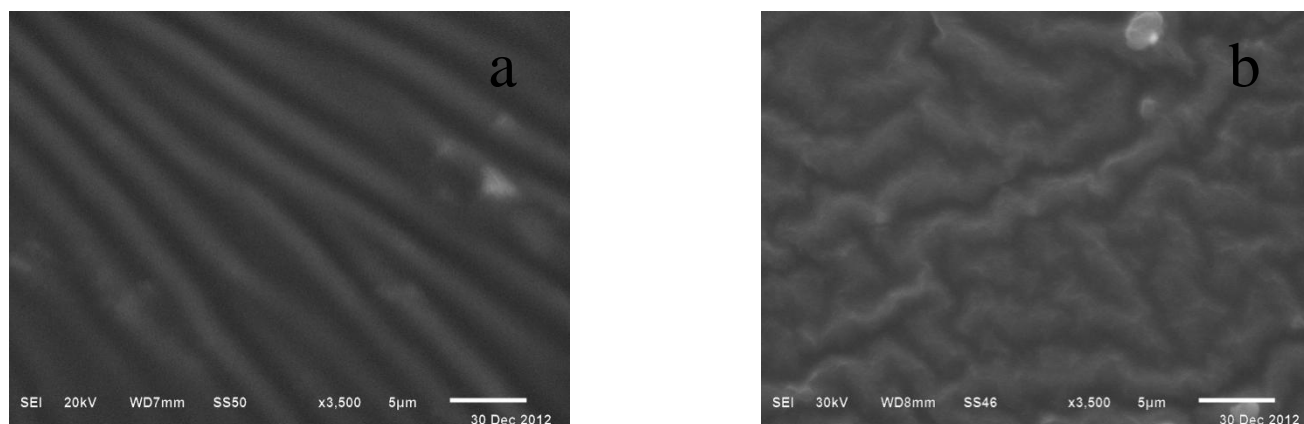


Fig.4. High-resolution electron micrographs of a membrane surfaces containing Pina-Rt ion pair. (a) Fresh membrane (3500-fold magnification), and (b) expired membrane (3500-fold magnification)

3.6 Influence of temperature

Thermal stability of the sensors was tested by constructing calibration graphs for the electrode potential, E_{elect} , versus $p\text{Pina}$ at different temperatures covering the range 20-60°C. The electrode potentials from these graphs at $p\text{Pina} = 0$ were obtained and plotted versus $(t-25)$, where t is the temperature of the solution. A straight line was obtained for each electrode and the slope (dE/dt) represents the thermal coefficient of the electrode^[18]. The slopes were found to be 0.00012, 0.00084 and 0.00083 V/°C for sensors no.5, 7 and 13, respectively. This indicates fairly high thermal stability of the sensors within the investigated temperature range and shows no deviation from the theoretical Nernstian behavior.

3.7 Influence of pH and effect of electrolytes

The effect of pH on the electrode potential at pinaverium bromide concentrations 1×10^{-4} and 1×10^{-3} mol L⁻¹ was studied. The pH was varied by adding HCl or NaOH solutions in different concentrations (each 0.1-1.0 mol L⁻¹). The results are shown in Fig. 5. As can be seen, the pH had little influence on the potential response, especially at high concentrations. The electrode response was checked with bidistilled water, 0.04 mol L⁻¹ Britton Robinson buffer pH 7.0 or 0.1 mol L⁻¹ phosphate buffer pH 7.0. The best results were achieved in 0.04 mol L⁻¹ Britton Robinson buffer pH 7.0 in the case of Pina-TPB sensor (sensor no.5), but in the case of the other sensors, using bidistilled water, it provided not only a higher Nernstian slope but also a stable potential reading. Therefore, 0.04 mol L⁻¹ Britton Robinson buffer pH 7.0 was used in the case of Pina-TPB sensor and bidistilled water was used in the case of the other sensors. The effect of ionic strength on the response of the sensors was also studied, by measuring the potential values of the sensors at different electrolyte concentrations, 0.01-1.0 mol L⁻¹ NaCl in 0.04 mol L⁻¹ Britton Robinson buffer pH 7.0 in the case of Pina-TPB sensor, and in bidistilled water in the case of the other sensors. The results indicate that there is no effect of ionic strength on the response of these sensors.

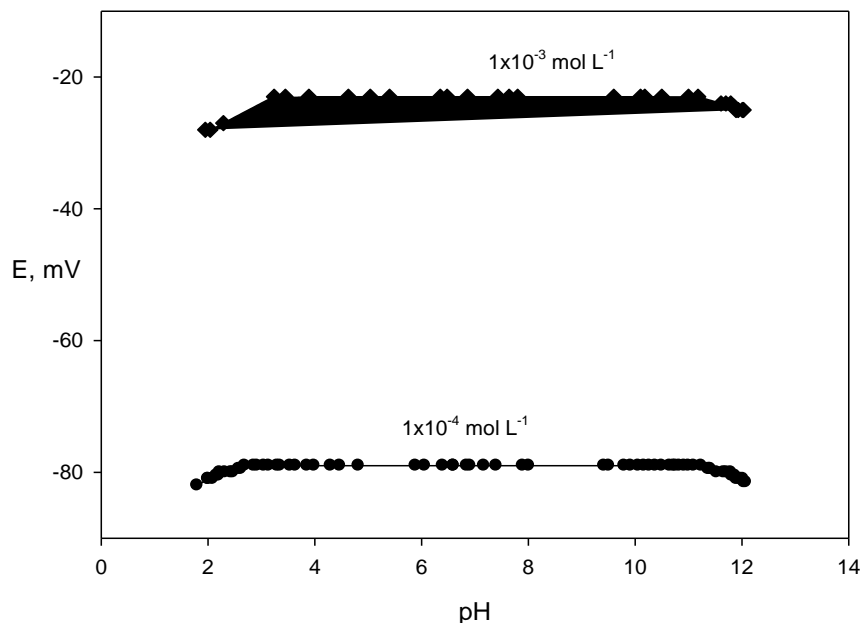


Fig. 5. Effect of pH on the potential responses of sensor no. 5

3.8 Determination of solubility products of Pina ion-pairs

Pungor and Toth^[19] have shown that the solubility products of the precipitates constituting the ion-selective sensors determine the detection limits of precipitate-based sensors. Hence the proposed electrodes were used for determination of the solubility products (K_{sp}) of pina-TPB and pina-RT ion-pairs by standard addition method. The solubility product of the pina-TPB and pain-RT ion-pairs were found to be 7.80×10^{-13} and 7.02×10^{-12} at 30°C , respectively indicating a relatively low solubility of ion pair containing NaTPB compared with that containing ammonium reineckate. This behaviour is in accordance with the difference in life span of the studied sensors.

4 Validation of the method

The linearity, limit of detection, selectivity, precision, accuracy, and ruggedness/robustness were the parameters used for the method validation.

4.1 Selectivity

Selectivity is an important characteristic of an electrode that delineates the extent to which the device may be used in the estimation of analyte ion in the presence of other ions and extent of utility of any electrode in real sample measurement. The selectivity of an ion-pair based membrane electrode depends on the physico-chemical characteristics of the ion-exchange process at the membrane. For example, sample solution interface, mobility of the respective ions in the matrix of the membrane and on the hydrophobic interactions between the primary ions and the PVC membrane^[20]. The selectivity of the Pinaverium membrane electrode is related to the free energy of transfer of the pinaverium cation between aqueous and membrane phases. None of the investigated species interferes, as shown by the very small values of the selectivity coefficient (Table 5). This reflects a very high selectivity of the investigated electrodes towards Pinaverium. The inorganic cations do not interfere because of the difference in their mobility and permeability as compared to pinaverium cation. In case of sugars and amino acids, the high selectivity is related to the difference in polarity and

lipophilic nature of their molecules relative to pinaverium cation. The mechanism of selectivity is mainly based on the stereo specificity and electrostatic environment and is dependent on how much fitting is present between the location of the lipophilicity sites in two competing species in the bathing solution side and those present in the receptor of the ion-exchanger ^[21].

Table 5. Selectivity coefficient values $K_{PinaJZ^+}^{pot}$ for Pina sensors

Interferent	Sensor no.5		Sensor no.7		Sensor no.13	
	SSM	MPM	SSM	MPM	SSM	MPM
Na ⁺	2.79x10 ⁻⁴	-	2.61 x10 ⁻³	-	3.47 x10 ⁻³	-
K ⁺	4.06x10 ⁻⁴	-	4.95 x10 ⁻³	-	7.00 x10 ⁻³	-
NH ₄ ⁺	5.42x10 ⁻⁴	-	3.83 x10 ⁻³	-	5.24 x10 ⁻³	-
Li ⁺	2.46 x10 ⁻⁴	-	1.71 x10 ⁻³	-	2.59 x10 ⁻³	-
Ca ²⁺	5.94 x10 ⁻⁵	-	1.70 x10 ⁻⁴	-	2.04 x10 ⁻⁴	-
Mg ²⁺	5.25 x10 ⁻⁵	-	1.77 x10 ⁻⁴	-	2.50 x10 ⁻⁴	-
Co ²⁺	8.99 x10 ⁻⁵	-	2.96 x10 ⁻⁴	-	3.08 x10 ⁻⁴	-
Cu ²⁺	1.06 x10 ⁻⁴	-	3.36 x10 ⁻⁴	-	3.49 x10 ⁻⁴	-
Mn ²⁺	1.54 x10 ⁻⁴	-	2.96 x10 ⁻⁴	-	4.29 x10 ⁻⁴	-
Fe ³⁺	1.74 x10 ⁻⁴	-	3.08 x10 ⁻⁴	-	3.79 x10 ⁻⁴	-
Vitamine B1	1.87 x10 ⁻²	-	1.84 x10 ⁻²	-	1.89 x10 ⁻²	-
Vitamine B6	8.35 x10 ⁻³	-	1.55 x10 ⁻²	-	1.60 x10 ⁻²	-
Glucose	-	8.47 x10 ⁻⁴	-	5.47 x10 ⁻⁴	-	9.22 x10 ⁻⁴
Fructose	-	9.22 x10 ⁻⁴	-	5.28 x10 ⁻⁴	-	4.51 x10 ⁻⁴
Maltose	-	6.47 x10 ⁻⁴	-	5.69 x10 ⁻⁴	-	4.94 x10 ⁻⁴
Lactose	-	7.16 x10 ⁻⁴	-	6.79 x10 ⁻⁴	-	5.47 x10 ⁻⁴
Urea	-	7.77 x10 ⁻⁴	-	6.47 x10 ⁻⁴	-	5.10 x10 ⁻⁴
β-alanine	-	7.57 x10 ⁻⁴	-	5.92 x10 ⁻⁴	-	5.69 x10 ⁻⁴
Glycine	-	9.22 x10 ⁻⁴	-	6.18 x10 ⁻⁴	-	5.28 x10 ⁻⁴

4.2 Ruggedness/ Robustness

For ruggedness ^[20] of the method a comparison was performed between the intra- and inter-day assay results for pinaverium obtained by two Ph.D. candidates. The RSD values for the intra- and inter-day assays of pinaverium in the cited formulations performed in the same laboratory by the two analysts did not exceed 2%. On the other hand, the robustness ^[22] was examined while the parameter values (pH of the eluent and the laboratory temperature) were deliberately slightly changed. Pinaverium recovery percentages were good under most conditions, not showing any significant change when the critical parameters were modified.

4.3 Accuracy

Accuracy is an important requirement of electroanalytical methods. It can be defined as the closeness between the true or accepted reference value and the obtained value ^[24]. The accuracy of the proposed method using the proposed sensors was investigated by the determination of pinaverium in spiked samples prepared from serial concentrations of pinaverium reference standards. The results summarized in Tables 6 and 7 show high accuracy of the proposed method, as indicated by the percentage recovery values.

4.4 Linearity

Under the optimal experimental conditions, linear relationships exist between the electrode potential/mV and the log [Pina]. The regression data, correlation coefficients (r^2) and other statistical parameter are listed in Table 2.

4.5 Limit of detection

LOD is the lowest quantity of the investigated compound in a sample that can be detected, but not necessarily quantified with an acceptable uncertainty. LOD of an electroanalytical method is an important factor if quantitative measurements are to be made at concentrations close to it. Especially, LOD is necessary for the trace analysis of drug active components in pharmaceuticals and/or human urine samples [23]. The values of LOD that are presented in Table 2 indicate that the sensors under investigation are highly sensitive, selective and can be applied in determination of small amounts of pinaverium bromide.

4.6 Precision

Precision is a measure of how close results are to one another. Precision is also expressed as the closeness of agreement between independent test results obtained under stipulated conditions. Precision is usually expressed as standard or relative standard deviations of the replicate analysis [23]. Hence the precision of the proposed potentiometric method using the sensors under investigation was measured as percentage relative standard deviation (RSD %) as shown in Tables 6 and 7.

Table 6. Determination of Pinaverium bromide in pure solution and pharmaceutical preparations applying the standard additions and potentiometric titrations method

Sample	Standard additions				Potentiometric titration			
	Taken mg	Found mg	Mean Recovery %	RSD %	Taken mg	Found mg	Mean Recovery %	RSD %
Sensor no.5								
Pure solution	5.91	5.89	99.00	0.98	44.35	44.06	99.93	0.95
	2.95	2.96	100.33	0.04	29.57	29.27	99.00	0.21
	2.36	2.33	98.72	0.03	14.78	15.08	102.03	0.83
	1.47	1.45	98.63	0.32				
Spascolon	5.91	5.83	98.64	0.87	44.35	44.06	99.93	0.53
	2.95	2.94	99.66	0.63	29.57	29.27	99.00	0.47
	2.36	4.50	98.00	0.32	14.78	15.08	102.03	0.37
	1.47	1.48	100.68	1.00				
Sensor no.7								
Pure solution	5.91	5.84	98.81	0.38	44.35	44.65	99.93	0.12
	2.95	2.94	99.66	0.99	29.57	29.86	99.00	0.80
	2.36	2.32	98.30	1.08	14.78	15.08	102.03	0.81
	1.47	1.46	99.31	1.30				
Spascolon	5.91	5.80	98.13	0.50	44.35	44.06	99.93	0.90
	2.95	2.93	99.32	0.99	29.57	29.27	99.00	0.75
	2.36	2.30	97.45	1.00	14.78	15.08	102.03	0.55
	1.47	1.44	97.95	0.92				

Sensor no.13

Pure solution	5.91	5.90	99.83	0.12	44.35	44.65	99.93	0.64
	2.95	2.93	99.32	1.23	29.57	29.86	99.00	0.42
	2.36	2.37	100.42	0.66	14.78	15.08	102.03	0.37
	1.47	1.45	98.63	0.97				
Spascolon	5.91	5.85	98.98	1.17	44.35	44.06	99.93	0.46
	2.95	2.93	99.32	0.89	29.57	29.27	99.00	0.69
	2.36	2.36	100.01	0.56	14.78	15.08	102.03	0.34
	1.47	1.48	100.68	0.73				

RSD: relative standard deviation (four determinations)

Table 7. Determination of pinaverium bromide in spiked urine samples applying the standard addition method

Sensor	Taken (mg)	Found (mg)	Recovery %	RSD %
Sensor no.5				
	5.91	5.88	99.49	1.01
	2.95	2.94	99.66	0.45
	2.36	2.37	100.42	0.75
	1.47	1.47	100.00	0.22
Sensor no.7				
	5.91	5.92	100.16	0.89
	2.95	2.93	99.32	0.34
	2.36	2.33	98.72	0.45
	1.47	1.48	100.68	1.09
Sensor no.13				
	5.91	5.85	98.98	1.95
	2.95	2.91	98.64	1.04
	2.36	2.31	97.88	0.55
	1.47	1.46	99.31	0.44

RSD: relative standard deviation (four determinations)

5 Analytical application

The accuracy and applicability of the proposed sensors were evaluated by its application for the determination of pinaverium bromide in pure solutions and in pharmaceutical preparations (Spascolon tablets) by both the standard addition and the potentiometric titration methods. Representative potentiometric titration curves and its first order derivatives are shown in Fig. 6.

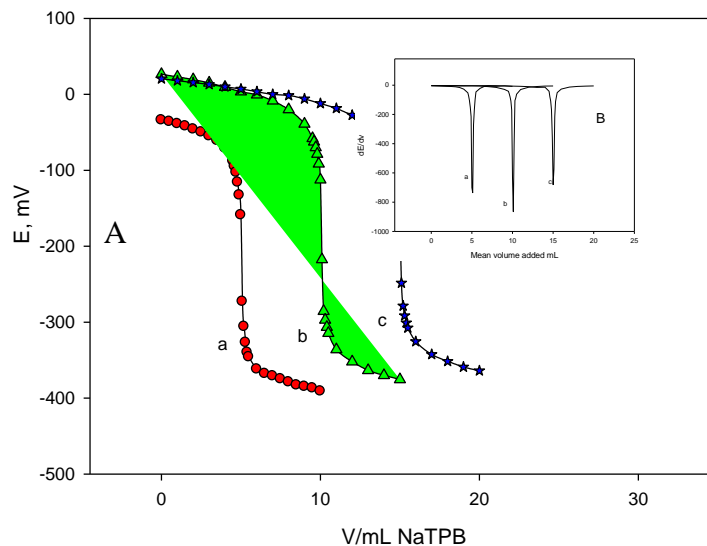


Fig. 6. Potentiometric titration curves (A) and its first order derivatives (B) of (a) 5, (b) 10 and (c) 15 mL of $5 \times 10^{-3} \text{ mol L}^{-1}$ Pina using sensor no. 13 and $5 \times 10^{-3} \text{ mol L}^{-1}$ NaTPB as titrant

The obtained average recovery and relative standard deviation values are summarized in Table 6, which reflect the high accuracy and precision of the sensors. Determination of pinaverium bromide in spiked urine samples was also carried at four different levels of concentration using the standard addition technique (Table 7). The proposed sensors can therefore be applied to the determination of pinaverium bromide in pure solutions, in pharmaceutical preparations, and in spiked urine samples without fear of interference caused by the excipients expected to be present in tablets or in the constituents of the body fluids. The results obtained from the standard addition method of the drug were compared with those obtained from the potentiometric titration method by applying F- and t-tests [24]. The results (Table 8) show that the calculated F- and t-values did not exceed the theoretical values, reflecting the accuracy and precision of the applied method.

Table 8. Statistical comparison between the results of an analysis of a pharmaceutical preparation Spascolon tablets applying the standard addition and potentiometric titration methods

Parameters	Standard addition method	Potentiometric titration method
		Sensor no.5
Mean recovery (%)	99.17 ^a	100.32 ^b
SD	0.789	1.267
RSD (%)	0.795	1.263
F-ratio	2.57 (9.55) ^c	
t-test	1.49 (2.57) ^d	
Sensor no.7		
Mean recovery (%)	99.02 ^a	100.32 ^b
SD	0.593	1.267
RSD (%)	0.599	1.263
F-ratio	4.56 (9.55) ^c	

t-test	1.84 (2.57) ^d	
Sensor no.13		
Mean recovery (%)	99.63	100.32 ^b
SD	0.627	1.267
RSD (%)	0.630	1.263
F-ratio	4.08 (9.55) ^c	
t-test	1.77 (2.57) ^d	

a: Average of four determinations

b: Average of three determinations

SD: standard deviation

RSD: relative standard deviation

c: Tabulated F-value at 95% confidence level

d: Tabulated t-value at 95% confidence level and six degrees of freedom

6 Conclusions

The present work involves the preparation of new PVC membrane sensors. The described sensors are sufficiently selective for the quantitative determination of pinaverium bromide in pure form, pharmaceutical dosage form, human urine. The present sensors show high sensitivity, reasonable selectivity, fast static response, long-term stability and applicability over a wide pH range with no sample pretreatment. The presented methods for the determination of pinaverium bromide with the prescribed sensors are advantageous over the previously described procedures being faster than many other techniques (response time of 10s). The suggested methods are characterized by low LOD values amounting to 10^{-6} mol L⁻¹ using sensors; thus they are more sensitive than many spectrophotometric and chromatographic methods. These sensors were used satisfactory for analyses of human urine without any interference from the matrix.

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