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RESEARCH ARTICLE

Improved Determination of Pinaverium Bromide in Pharmaceutical Preparations and Human Urine Utilizing a Coated Wire Electrode

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Abstract

Our efforts to lower the detection limit for a pinaverium ion-selective electrode were presented. Coated graphite CGE and coated silver CSE electrodes for pinaverium ions based on pinaverium-tetraphenylborate (pina-TPB) and pinaverium-reineckate (pina-RT) as electroactive materials are described. The sensors show Nernstian slopes of 59.5±0.4 and 59.2±0.2 mV decade^{-1} with detection limits of 6.3×10^{-7} and 6.3×10^{-7} mol L^{-1} pinaverium bromide for CGE and CSE, respectively. Furthermore, the sensors showed invariable potentials in the pH range of 3-11 for CGE and CSE. The present electrodes exhibit clear discrimination of pinaverium bromide from several inorganic cations, sugars and amino acids. The sensors have been used for the determination of pinaverium bromide in human urine and pharmaceutical preparations via potentiometric titration, standard addition and the calibration curve methods. The results were acceptable with excellent accuracy and precision.

Introduction

Ion-selective electrodes (ISEs) were found to have extensive uses for the direct determination of several ionic species [1-11]. In traditional polymeric membrane ion-selective electrodes (ISEs), the sensing membrane is interjected between two aqueous phases, the inner solution and the sample. However; these electrodes still have definite inherent limitations. They are mechanically intricate, and thus difficult to be fabricated in small size. Besides, the flux of ions from the membrane that is in contact with the inner electrolyte solution containing a salt of the primary ion, toward the sample makes the concentration in the contacting aqueous layer be circa 10^{-6} mol L^{-1}. Therefore, the lower detection limit was come to be nearly10^{-6} mol L^{-1} [12-14]. One approach to surpass this drawback is removal of the inner solution by using a solid inner contact. In a solid-contact or ‘‘coated wire’’ ISE, the polymer membrane is casted in a direct way on the solid surface, without internal reference solution being interjected. An exciting advance was achieved in ISEs by Cattrall and Freiser [15] when they started to develop coated wire ISEs. CWEs may suffer from problems of being reproducible and stability on the long-term (drifting potential), ensuing from the poorly defined contact and mechanism of charge-transfer between the membrane coating and the conducting transducer. Coated electrodes where an electroactive species is incorporated into a thin polymeric film coated directly on a metallic or graphite conductor has been evinced to be very effective for a variety of inorganic and organic cations and anions [16-21]. Electrodes of this species are simple, cheap, long lasting, capable of reliable response in a wide concentration range for a wide variety of both organic and inorganic ions and

suitable for measurements in small volumes of sample or for the desirable in vivo applications of ISEs that biomedical researchers have awaited for a long time.

Pinaverium bromide, [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)] an L-type calcium channel blocker, being selective for the gastrointestinal tract, effectively puts an end to pain, diarrhea and intestinal discomfort, makes available good therapeutic efficacies without considerable adverse effects on IBS patients [22-25]. Pinaverium can be found as tablets at the dose of 50 and 100 mg/tablet in the market with the brand name Spascolon®. Pinaverium exhibits a low absorption (8-10%), and maximum blood levels are peaked after 1 h of oral administration. Some 97% of the drug is bounded to protein in the plasma. Consequently, we weren't able to determine the pinaverium bromide in human plasma in our work. With 1.5 h mean half-life, it goes through a first-pass metabolism reducing the bioavailability at therapeutic doses. Pinaverium bromide is almost removed after transformation is done in the liver [26, 27].

Several methods have been mentioned for the identification of pinaverium bromide in dosage forms and in biological fluids as follows: liquid chromatography-electrospray tandem mass spectrometry [27], high-performance liquid chromatography [28-29], gas chromatography mass spectrometry [30], but these methods have a variety of drawbacks, i.e. they are neither cheap nor appropriate for monitoring on a large-scale.

This work gives a description of construction and investigation of performance characteristics of new ISEs based on coated wire electrodes for the determination of pinaverium bromide. With the current electrode, a drop in the detection limit by an order of magnitude was reached (from 10^{-6} to 10^{-7} mol L^{-1}). These potentiometric sensors were found to give accurate results for the determination of pinaverium bromide in mass powder, pharmaceutical formulations and human urine.

2. EXPERIMENTAL

2.1. Reagents and materials

All chemicals were analytical grade reagents (AR). Double distilled water is used during all experiments. Pure grade pinaverium bromide and the pharmaceutical preparation Spascolon® tablets (100 mg/tablet) were supplied by Chemipharm Pharmaceutical Industries, 6th October City-Egypt. Sodium tetraphenylborate (NaTPB), ammonium reineckate (NH_4RT), poly (vinyl chloride) with high molecular weight (PVC), tetrahydrofuran (THF) and dibutyl phthalate (DBP) were bought from Merck (Germany). The metal salts were provided by BDH Company (UK) in forms of either nitrates or chlorides, besides stock solutions of the metal salts were prepared in the form of bi-distilled water and standardized when-ever necessary.

2.2. Apparatus

Potentiometric and pH-measurements were conducted using 702 titroprocessor equipped with a 665 dosimat (Switzerland) made by Metrohm. A mLw W20 circulator thermostat was in use for the sake of controlling the temperature of the test solutions. A saturated calomel electrode (SCE) was used as the external reference. The electrochemical systems may be represented as following: graphite rod //membrane/test solution//KCl salt bridge// SCE and metal wire (silver, copper and aluminum)//membrane/test solution//KCl salt bridge// SCE

2.3. Preparation of the ion-pairs

The ion-pairs (Pina-TPB and Pina-RT) were prepared by making a mixture of 100 mL 10^{-2} mol L^{-1} pinaverium bromide solution with 100 mL of 10^{-2} mol L^{-1} of sodium tetraphenylborate or ammonium reineckate. The formed precipitates were subject to filtration and complete washing with cold bi-distilled until bromide free (tested using $AgNO_3$ solution) and then drying at room temperature. The composition of the ion-pair was 1: 1 both in case of Pina-TPB and Pina-RT as confirmed by elemental analysis data reported in our recently published paper [31]. The percentage values found are 71.89, 7.38 and 1.86 and those calculated 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. Electrode preparation

2.4.1. Coated wire electrode

CWEs were prepared with the use of silver, copper and aluminum metal wires (1 mm diameter) and graphite rod (4 mm diameter) based on the procedures stated in detail elsewhere [32]. The polished electrodes were dipped in the coating solution (a solution of the same best membrane composition which was used for the membrane type) and allowed to dry in air for about 1 min to produce a film of the coating material. The process was reiterated till a membrane film nearly 1 mm thick was formed (about 8 times). The prepared electrode was soaked as a precondition for 30 min in 10^{-3} mol L^{-1} pinaverium bromide solution.

2.5. Electrodes calibration

The conditioned electrodes were measured by separately transferring 50 mL aliquots of solutions ($1.0x10^{-7}$ to $5.0x10^{-3}$ mol L^{-1}) of pinaverium bromide into a series of 100-mL beakers. The CW electrodes, in synchronization with saturated calomel electrode, were dipped in the abovementioned test solutions and permitted to equilibrate while they were being stirred. The potential was recorded after being stabilized to ±1 mV, and the potential was regarded as a function of the negative logarithm of pinaverium bromide concentration.

2.6. Selectivity coefficient determination

The separate solution method and the matched potential method (MPM) [33-37] were used in determination of the selectivity coefficients, $\log K^{pot}_{Pina,J^{z+}}$, of the potentiometric sensors regarding different species. During a separate solution method, the potential of a cell comprising a working electrode and a reference electrode is calibrated in two separate solutions, where, E_1 is the potential measured in 1.0×10^{-3} mol L^{-1} Pina, E_2 the potential measured in 1.0×10^{-3} mol L^{-1} of the interfering compound, z_1 and z_2 are the charges of pinaverium and interfering species, successively and S is slope of the electrode calibration plot, then the selectivity coefficients were determined via the separate solution method using the rearranged Nicolsky equation:

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

In 1995, IUPAC recommendation [35] prescribes the matched potential method (MPM) [36, 37] as the method of choice for ions of different charge. MPM is considered [35] as a purely operational method, not depending on any theoretical or empirical, model equation. The value used to express the extent of interference is the ratio of primary ion concentration increment to the interfering ion concentration that gives the same potential change in a constant initial background of primary ion. The selectivity factors of the electrodes were determined by MPM. In accordance with this method, the activity of Pina solution was being on increase from $a_A = 5.0x10^{-5}$ mol L^{-1} (reference solution) to $\grave{a}_A=6.0x10^{-5}$ mol L^{-1}, and the changes in potential (ΔE) corresponding to this increase were calibrated. Next, a solution of an interfering ion of concentration a_B in the range $1.0x10^{-1}$-$1.0x10^{-2}$ mol L^{-1} was added to new $5.0x10^{-5}$ mol L^{-1} pina solution till the same potential change (ΔE) was recorded, then the selectivity factor $K^{pot}_{Pina,J^{z+}}$ for each studied species was calculated using the following equation:

$$K^{pot}_{Pina,J^{z+}} = \frac{(\grave{a}_A - a_B)}{a_B}$$

2. 7. Potentiometric determination of pinaverium bromide

The standard addition method was applied [8, 38], in which small increments of the standard solution $5.0x10^{-3}$ mol L^{-1} of pinaverium bromide were added to 50 mL aliquot samples of various concentrations from pure drug or pharmaceutical preparations. The change in millivolt reading was recorded for each increment and used to calculate the concentration of pinaverium bromide sample solution using the following equation:

$$C_x = C_s \left(\frac{V_s}{V_x+V_s}\right) \left(10^{n\,(\Delta E/S)} - \frac{V_X}{V_x+V_s}\right)^{-1}$$

Where: C_x and V_x are the concentration and the volume of the unknown, respectively, C_s and V_s the concentration and the volume of the standard solution, respectively, S the slope of the calibration graph and ΔE is the change in mV due to the addition of the standard solution.

2. 8. Potentiometric titration of pinaverium bromide

An aliquots of $5.0x10^{-3}$ mol L^{-1} drug solution (pure or tablet), were transferred into 50 mL volumetric flasks and made up to the mark with bi-distilled water. Different concentrations of pinaverium bromide were prepared, then titrated potentiometrically with a standard solution of $5.0x10^{-3}$ mol L^{-1} NaTPB. The volume of the titrant at equivalence point was obtained using the S-shaped titration curves. The differential graphs of the titration curves have also been constructed to obtain well defined and sharp end points using the computer program Sigma plot [8].

2.9. Determination of pinaverium bromide in spiked urine samples

Different amounts ($5.0x10^{-5}$-$2.0x10^{-4}$ mol L^{-1}) of pinaverium bromide and 5 mL urine of a healthy person were transferred to a flask measuring 50 mL and completed to reach the mark with the use of bi-distilled water. The contents of the measuring flask were transferred to a 100 mL beaker, and were subject to potentiometric determination of pinaverium bromide with the aid of the standard addition method.

Result and Discussion

Table.1. Optimization of membrane compositions and their potentiometric response for coated wire pinaverium selective electrodes

Sensors No.	Composition of membrane% (w/w; mg)					Slope	Linear concentration range (mol L^{-1})	LOD (mol L^{-1})	LOQ (mol L^{-1})	RSD %
	Pina-TPB	Pina-RT	PVC	DBP	Electrode bed	mV/decade				
1	12	-	44	44	Graphite	59.5±0.4	5.0×10^{-6}–5.0×10^{-3}	6.3×10^{-7}	2.1×10^{-6}	0.67
2	12	-	44	44	Silver	5**8.3**±0.3	5.0×10^{-6}–1.0×10^{-3}	5.0×10^{-7}	1.7×10^{-6}	0.51
3	12	-	44	44	Copper	57.1±0.4	5.0×10^{-6}–1.0×10^{-3}	1.3×10^{-6}	4.2×10^{-6}	0.70
4	12	-	44	44	Aluminum	31.5±0.5	5.0×10^{-6}–1.0×10^{-3}	1.0×10^{-6}	3.3×10^{-6}	1.58
5	-	1	49.5	49.5	Graphite	57.0±0.4	1.0×10^{-5}–1.0×10^{-3}	1.6×10^{-6}	5.3×10^{-6}	0.70
6	-	1	49.5	49.5	Silver	59.2±0.2	5.0×10^{-6}–1.0×10^{-4}	6.3×10^{-7}	2.1×10^{-6}	0.34
7	-	1	49.5	49.5	Copper	56.8±0.3	5.0×10^{-6}–1.0×10^{-4}	1.0×10^{-6}	3.3×10^{-6}	0.53
8	-	1	49.5	49.5	Aluminum	49.7±0.5	5.0×10^{-6}–1.0×10^{-4}	1.6×10^{-6}	5.3×10^{-6}	1.01

LOD: limit of detection
LOQ: Limit of quantitation
RSD: relative standard deviation (four determinations)

The electrode response depends upon the concentration gradient of pinaverium ions across the membrane as well as on the properties of the polymer matrix, amount of the electroactive material, plasticizer and its fabrication. The electrodes with different amounts of these components are assessed for their characteristic parameters [39] and the results are given in Table 1.

3.1. Optimization of membrane composition

Electroactive materials were in use for construction of ion-selective membrane sensor should exhibit rapid exchange kinetics and possible stability. Besides, they should have discernible solubility in the membrane matrix and adequate lipophilicity; in order to prevent leaching from the membrane into the sample solution [40- 42]. Electroactive materials added to each electrode were an ion-association of the drug cation with an anion; tetraphenylborate or reineckate. These sorts with high molecular weight anions have various lipophilicities and stabilities; in addition they were in use as electro-active materials in coated electrode construction and were candidates for the formation of highly lipophilic ion-pairs as well as active recognition elements in the proposed electrodes. Consequently, the variation in potential with different amounts of ion-pairs was reviewed in our published paper [31]. The best performance was conducted via (Pina-TPB, DBP and PVC in ratio 12:44:44, respectively. This sensor indicated a nearly Nernstian response with slope of 57.6±0.2 mV $decade^{-1}$ and a linear concentration range $5.0x10^{-6}$ -$5.0x10^{-3}$ mol L^{-1} and limit of detection was $2.0x10^{-6}$ mol L^{-1}) and Pina-RT, DBP and PVC in ratio 1:49.5:49.5, successively, demonstrated an approximately Nernstian response with slope of 53.6± 0.4mV/decade as well as a linear concentration range $9.8x10^{-6}$ -$5.0x10^{-3}$ mol L^{-1} and limit of detection was found to be $5.0x10^{-6}$ mol L^{-1}) [31].

The above results sustain the notion that ion-pair complexes with higher molecular mass have lower solubility and will result in lower detection limit.

Plasticizer exerts a vital role in the sensor performance; due to their responsibility for ion-pair solvation and distribution in the membrane matrix, consequently controlling the detection limit which affects the selectivity and sensitivity plus giving the plastic membrane to its proper elasticity and strength. Polar plasticizers lead to the membrane resistance reduction as compared with nonpolar plasticizers containing other functional groups with potential coordination sites which might compete with the carrier [43, 44]. The plasticizers have an influence upon the dielectric constant of the membrane phase, ensure high mobility of ions in the membrane [45-47] and it is expected to play a vital role in the characteristics of the ion-selective electrode. Thus, the influence of the plasticizer type on the characteristics of the pinaverium-sensors was checked by using four plasticizers with different polarities, like DBP, DOP, DOS, and TCP. The results demonstrate that DBP is the best plasticizer put on test [8, 31]. Poor sensitivities for the electrodes plasticized using DOP, DOS and TCP are because of low solubilities or low distributions of Pina-TPB and Pina-RT ion-pairs in these solvents [48]. The electrodes using DBP as a plasticizer

give higher slopes, wide response range, more stable potential reading and lower limit of detection due to the optimal extraction of the drug in the organic layer of the membrane [49, 50].

The effect of the bed nature on efficacy of coated wire electrodes was examined. The improved coating mixture was utilized for preparation of electrodes with different conductive beds, i.e. silver, copper, graphite and aluminum. After providing the conditions, each electrode was checked in the concentration range from $1.0x10^{-7}$-$5.0x10^{-3}$ mol L^{-1} pinaverium bromide. The linear range of concentration and the limit of detection of the electrodes were assessed according to the IUPAC recommendations [42, 51]. According to the table 1, the graphite Pina-TPB (sensor No. 1) (CGE) and the silver Pina-RT (sensor No. 6) (CSE) provides the optimal response towards pinaverium cation [slope 59.5±0.4 and 59.2±0.2 mV $decade^{-1}$ at 25±0.1°C and detection limit 6.3×10^{-7} and 6.3×10^{-7} mol L^{-1} pinaverium bromide, respectively] for Pina-TPB and Pina-RT ion-pairs, respectively, (Fig. 1).

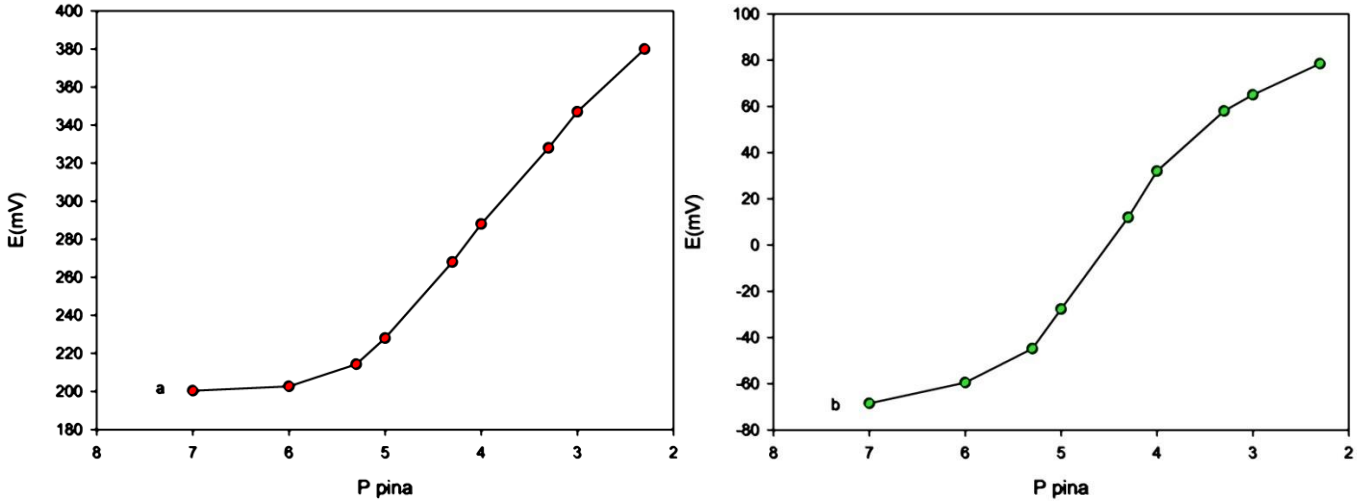

Figure 1. Calibration curves for pinaverium electrodes: (a) CGE, and (b) CSE.

The improved performance characteristics of the coated wire electrode in comparison with those of polymeric membrane electrode supposedly ensues from the coated wire technology, where an internal solution, in case of conventional liquid contact electrode, has been substituted by a wire of much higher electrical conductivity. It can be noticed that in case of electrodes based on Pina-RT all wires give low response towards pinaverium bromide in comparison with that of silver wire. Ag wire-coated electrode CSE has a slope 59.2±0.2 mV $decade^{-1}$ and a detection limit of 6.3×10^{-7} mol L^{-1}. This is attributable to low resistivity (1.62 $\mu\Omega cm^{-1}$) of silver [52], while in case of electrodes based on Pina-TPB all wires have low response towards pinaverium bromide in comparison to that of graphite rod. Graphite rod-coated electrode CGE has a slope 59.5±0.4 mV$decade^{-1}$ and a detection limit of 6.3×10^{-7} mol L^{-1}. This is attributed to the higher electrical conductivity of graphite rod. Therefore, silver wire and graphite rod were used as the inner solid contact for the electrodes in this study.

3.2. Effect of soaking

The lifetime of the electrodes was decided by soaking both CGE and CSE in 1.0×10^{-3} mol L^{-1} a solution containing pinaverium bromide for different interval until the electrode lost its Nernstian behavior, as a result this behavior can be attributable to the decomposition of the ion-pair and loss of other components in the membrane phase in contact with aqueous test solution which contains the pinaverium cation. The response of the electrodes has been measured by recording the calibration graph at 25 °C at different intervals. The results showed that the lifetimes measured in this way were found to be 8 and 3 days for CGE and CSE, respectively. The life spans of the coated wire electrodes, in general, are less than those of the corresponding liquid contact electrodes due to the probability of the poor mechanical adhesion of the PVC-based sensitive layer to the conductive bed [42, 53, 54]. It is worth mentioning that the short life time of the polymeric membrane electrodes were reviewed in our recently published paper using the scanning electron microscope [31].

3.3. Response time, reversibility and reproducibility

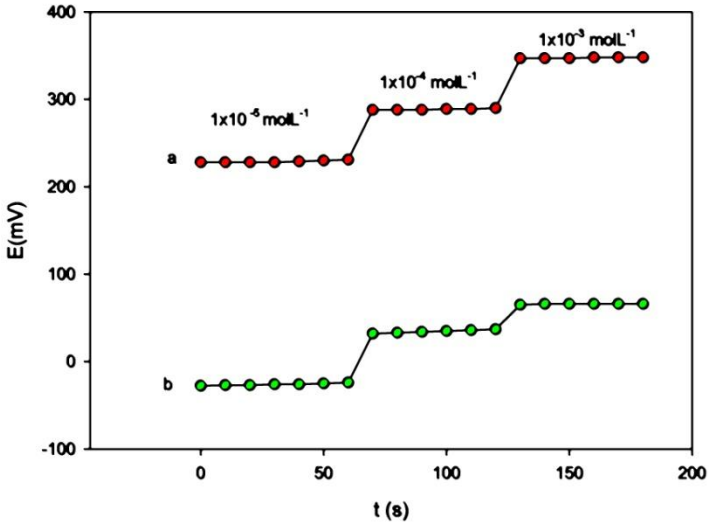

Figure 2. Dynamic response time for pinaverium electrodes: a) CGE; and (b) CSE.

Dynamic response time is a significant factor, for the assessment of any sensor. To measure the dynamic response time of the proposed electrodes the concentration of the test solution was consecutively changed from 1.0×10^{-5} to 1.0×10^{-3} mol L^{-1}, so the resulting data indicate that the time required reaching a potential within ±1 mV of the final equilibrium value after successive immersion of a series of pinaverium bromide solution, each having a 10-fold difference in concentration is 10 s for CGE and CSE as stated in Fig. 2, besides the repeatability of the potential reading for each electrode was investigated by following measurement in 1.0×10^{-3} mol L^{-1} solution shortly after measuring the first set of solutions at 1.0×10^{-4} mol L^{-1} [44]. The S.D. of 10 reproduce measurements at 1.0×10^{-3} and 1.0×10^{-4} mol L^{-1} pinaverium bromide were ±0.2 and ±0.3 mV for CGE and CSE, respectively. This shows the optimal repeatability of the potential response of the electrodes as stated in Fig. 3. .

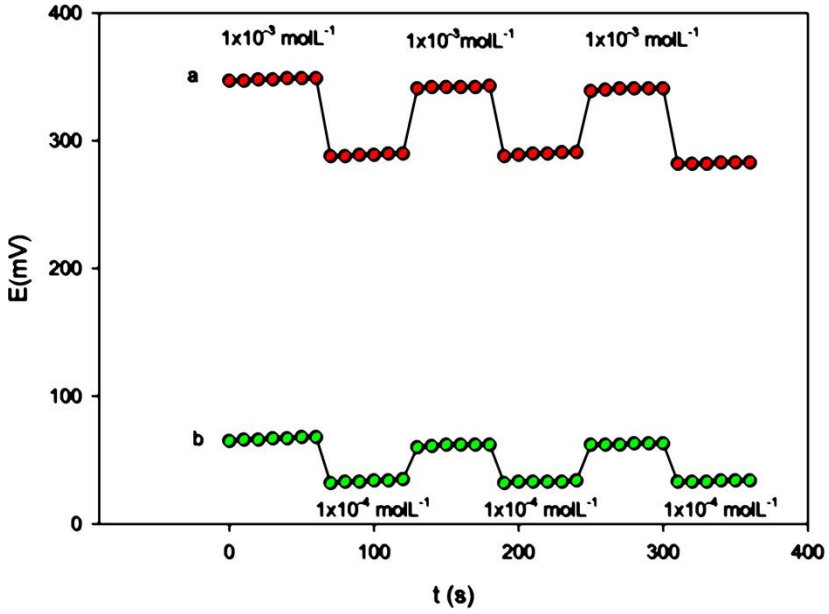

Figure 3. Dynamic response time of pinaverium electrodes: a) CGE; and (b) CSE for several high-to-low sample cycles.

3.4. pH *dependence*

The influence of pH on the response of both CGE and CSE was checked at 1.0×10^{-3} and 1.0×10^{-4} mol L^{-1} pinaverium bromide solution. The pH of the solution was changed by small addition of 0.1 mol L^{-1} solution of either HCl or NaOH and the results are shown in Fig. 4. The potential pH profile obtained indicates that the responses of the two electrodes were fairly constant over the pH range 3-11 for CGE and CSE.

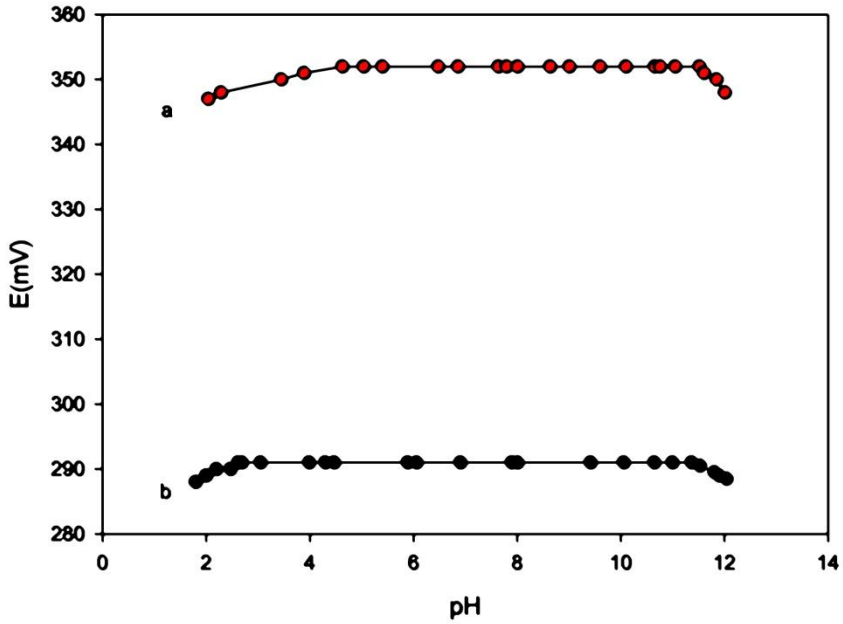

Figure 4. Effect of pH of the test solution on the potential response of the CGE electrode: (a) $1.0x10^{-3}$ mol L^{-1} pinaverium bromide; (b) 1.0×10^{-4} mol L^{-1} pinaverium bromide.

3.5. Selectivity of the electrode

The selectivity behavior is clearly one of the most salient features of an ion-selective electrode, identifying whether a reliable measurement in the target sample is possible. The selectivity coefficients for pinaverium bromide with regard to a variety of inorganic cations, sugars, amino acids, vitamins and urea were determined by the separate solution method (SSM) and the matched potential method (MPM). The resulting selectivity coefficients for the proposed electrodes are briefed in Table 2

It is immediately clear that the proposed electrodes are greatly selective towards pinaverium bromide. The inorganic cations do not interfere owing to the difference in their ability to move and to permeate in comparison with pinaverium cation. The selectivity sequence considerably differs from the so called Hofmeister selectivity sequence [55] (i.e. selectivity, merely based on lipophilicity of cations). In case of sugars and amino acids, the high selectivity is in relation to the difference in polarity and lipophilic nature of their molecules relative to pinaverium bromide. The mechanism of selectivity is basically based on the stereospecificity and electrostatic environment, and counts on how much fitting is available between the locations of the lipophilicity sites in two competing species in the bathing solution side and those present in the receptor of the ion-exchanger [56].

3.6. Analytical applications

The proposed electrodes were found to be working well under laboratory conditions. It can be observed that the amount of pinaverium bromide can be precisely decided via the proposed electrodes. In order to evaluate the applicability of the proposed electrodes, pinaverium bromide was measured in pure solutions, pharmaceutical preparations (Spascolon® tablets) and spiked urine samples, via these electrodes by the standard method of addition. The received average recovery and relative standard deviation values are briefed in Tables 3, and 4 reflecting the high accuracy and precision of the electrodes. The improved pinaverium bromide selective electrodes were successfully applied as indicator electrodes in the potentiometric titration of pinaverium bromide solution with NaTPB solution (Table 3).

The well-defined potential jumps of the titration curves (Fig. 5) are in correspondence with the formation of a Pina-TPB complex of 1:1 stoichiometry indicating the high sensitivity of the electrodes. Clearly, the two methods, standard addition and potentiometric titration, can be applied to the identification of pinaverium bromide in large volume of powder and in pharmaceutical formulations or in human urine without interference by the excipients which are expected to be available in tablets or the constituents of body fluids.

Table 2. Selectivity coefficient values of the pinaverium- selective electrodes

$K^{pot}_{Pina,J^{Z+}}$				
Interferent	CGE		CSE	
	SSM	MPM	SSM	MPM
Na^+	9.22×10^{-5}	----	7.24×10^{-5}	----
K^+	1.68×10^{-4}	----	2.52×10^{-3}	----
NH_4^+	3.26×10^{-4}	----	2.34×10^{-3}	----
Li^+	2.52×10^{-5}	----	8.75×10^{-5}	----
Fe^{2+}	8.39×10^{-5}	----	4.95×10^{-5}	----
Ca^{2+}	4.68×10^{-5}	----	2.34×10^{-5}	----
Mg^{2+}	2.73×10^{-4}	----	1.09×10^{-4}	----
Mn^{2+}	1.12×10^{-4}	----	1.89×10^{-4}	----
Cu^{2+}	8.29×10^{-5}	----	1.26×10^{-4}	----
Co^{2+}	4.37×10^{-5}	----	1.87×10^{-4}	----
Vitamine B1	----	1.64×10^{-2}	----	1.56×10^{-2}
Vitamine B6	----	2.12×10^{-2}	----	1.35×10^{-2}
Glucose	----	6.23×10^{-5}	----	5.35×10^{-5}
Fructose	----	4.44×10^{-5}	----	3.36×10^{-5}
Lactose	----	3.52×10^{-5}	----	4.89×10^{-5}
Maltose	----	8.12×10^{-5}	----	7.30×10^{-5}
Urea	----	2.55×10^{-5}	----	3.79×10^{-5}
Glycine	----	5.33×10^{-5}	----	4.62×10^{-5}
β-alanine	----	2.24×10^{-5}	----	3.11×10^{-5}

Table 3. Determination of pinaverium bromide in pure solutions and pharmaceutical preparations applying the standard addition and the potentiometric titration methods

Sample	Standard addition method			Potentiometric titration method		
	Taken (mg)	Recovery (%)	RSD (%)	Taken (mg)	Recovery (%)	RSD (%)
CGE						
Pure solutions						
	5.91	99.23	1.06	17.74	98.89	0.88
	2.95	99.16	1.31	26.61	99.56	1.03
	2.36	98.25	0.98	35.48	99.29	1.12
	1.47	99.89	0.91			
Spascolon® (100 mg/tablet)						
	5.91	99.83	0.87	17.74	100.23	0.69
	2.95	98.45	1.89	26.61	98.38	0.39
	2.36	100.03	0.65	35.48	99.67	1.76
	1.47	99.25	1.07			
CSE						
Pure solutions						
	5.91	100.25	0.96	17.74	99.89	0.93
	2.95	99.87	0.86	26.61	99.33	0.34
	2.36	99.94	0.75	35.48	100.07	1.06
	1.47	100.97	1.94			
Spascolon® (100 mg/tablet)						
	5.91	100.73	0.46	17.74	99.59	0.62
	2.95	98.33	0.38	26.61	99.35	0.78
	2.36	100.22	1.91	35.48	101.04	1.56
	1.47	99.57	0.82			

Table 4. Determination of pinaverium bromide in spiked urine samples applying the standard addition method

Sample	Spiked Urine		
	Taken (mg)	Recovery (%)	RSD (%)
CGE			
	5.91	98.98	0.27
	2.95	98.64	0.42
	2.36	97.88	0.35
	1.47	97.27	1.99
CSE			
	5.91	99.15	0.95
	2.95	98.64	0.74
	2.36	98.72	0.85
	1.47	97.96	0.24

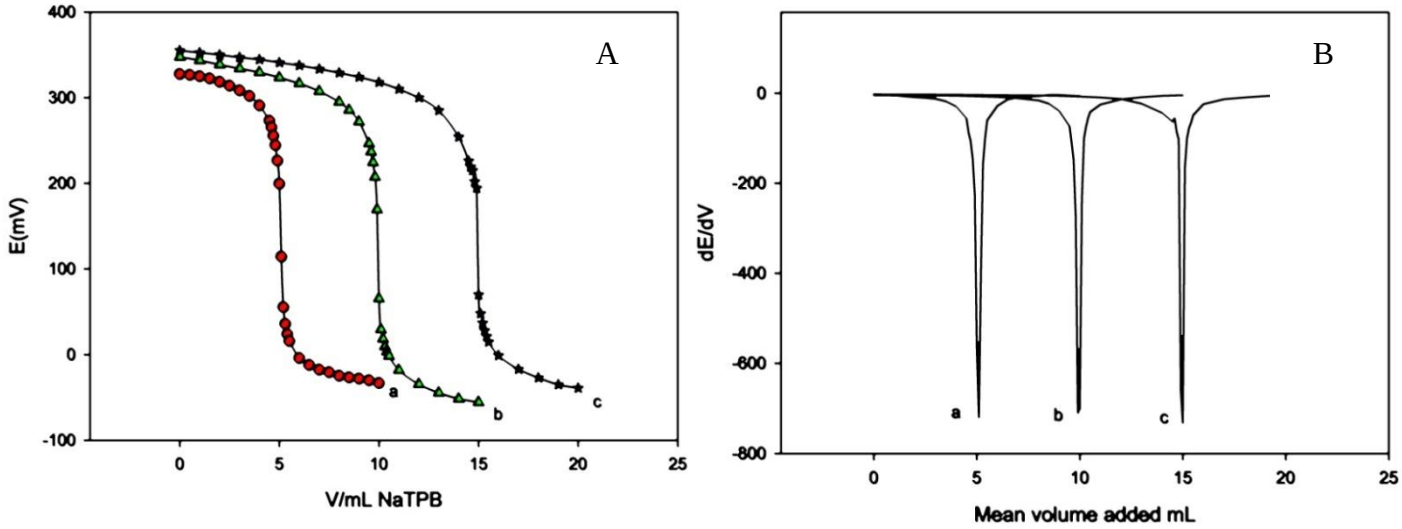

Figure 5. Potentiometric titration curves (A) and its first derivative (B) of (a) 5, (b) 10 and (c) 15 mL of $5.0x10^{-3}$ mol L^{-1} pinaverium bromide using CGE electrode and $5.0x10^{-3}$ mol L^{-1} NaTPB as titrant.

Table 5. Statistical comparison between the results of an analysis of a pharmaceutical preparation applying the standard addition and potentiometric titration methods

Parameters	Standard addition method	Potentiometric titration method
CGE		
Mean recovery (%)	99.39 [a]	99.42 [b]
SD	0.70	0.90
RSD (%)	0.71	0.91
F-ratio	1.65 (9.55)[c]	
t-test	0.05 (2.57)[d]	
CSE		
Mean recovery (%)	99.71[a]	99.99 [b]
SD	1.03	0.91
RSD (%)	1.04	0.92
F-ratio	1.28 (9.55) [c]	
t-test	0.37 (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 five degrees of freedom

3.7. Statistical analysis and validity of the proposed method

The linearity, limit of detection, accuracy, precision, and ruggedness or robustness were the factors used for the method validation. As previously mentioned, the measuring range of the pinaverium electrodes is 1.0×10^{-5}-5.0×10^{-3} mol L^{-1} pinaverium bromide.

3.7.1. Ruggedness

As to ruggedness of the method, a comparison was held between the intra- and inter-day assay results for pinaverium which were taken via two Ph.D. candidates. The RSD values for the intra- and inter-day assays of pinaverium in the mentioned formulations which were performed in the same laboratory by the two analysts did not surpass 1.99% indicating that the method is able to produce results with high accuracy.

3.7.2. Robustness

The robustness was checked while the parameter values (pH of the medium and the laboratory temperature) were being intentionally partly changed. Pinaverium recovery percentages were good under most conditions; i.e., they didn't show any significant change when the critical parameters were modified.

The results obtained from the standard addition method of the drug were in comparison with those obtained from the potentiometric titration method by applying F-and t-tests [57]. The results (Table 5) indicate that the calculated F- and t-values did not surpass the theoretical values. This, in turn, reflects the accuracy and precision of the applied method.

Table 6. Comparison between the suggested and some of the other published methods for determination of pinaverium bromide.

Method	Linear range mol L^{-1}	LOD mol L^{-1}	Slope	r^2	RSD%	Ref
Liquid chromatography						
liquid chromatography-electrospray tandem mass spectrometry	$3.7x10^{-11}$-$3.7x10^{-8}$	$3.7x10^{-11}$	-----	0.9979	less than15	[27]
high performance liquid chromatographic	$3.4x10^{-5}$-$2.5x10^{-4}$	$3.8x10^{-8}$	-----	0.9999	less than 2	[28]
Ion-Selective Electrode						
Pina-TPB (PME)	5.0×10^{-6} -5.0×10^{-3}	$2.0x10^{-6}$	57.6±0.2	0.9999	0.37	[31]
Pina-RT (PME)	9.8×10^{-6} -5.0×10^{-3}	$5.0x10^{-6}$	53.6±0.3	0.9994	0.69	[31]
CGE	5.0×10^{-6} -5.0×10^{-3}	6.3×10^{-7}	59.5±0.4	0.9998	0.67	[P.S]
CSE	5.0×10^{-6} -1.0×10^{-4}	6.3×10^{-7}	59.2±0.2	0.9997	0.34	[P.S]

r^2: Correlation coefficient
P.S: Present study
RSD: relative standard deviation
LOD: limit of detection
PME: Polymeric membrane electrode

4. CONCLUSION

The proposed pinaverium coated wire electrode based on Pina-TPB and Pina-RT as electroactive materials might be a useful analytical tool and a useful alternative for the determination of pinaverium ions in pharmaceutical formulations and human urine samples. It was evinced here that the selectivity behavior of the present coated wire

electrodes has witnessed a very good improvement in comparison with the conventional liquid contact electrodes. Based upon the results obtained with our electrodes, it becomes clear that the prepared electrodes exhibited good operating characteristics including reasonable detection limit, high selectivity, wide dynamic range and fast response for pinaverium bromide determination. These characteristics and the typical applications presented in this paper make the electrodes suitable for measuring pinaverium bromide content in pharmaceutical samples without a significant interaction from concomitant substances.

This study was compared with some previously published data. The results of this study indicated a wider linear range, $5.0x10^{-6}$-$5.3x10^{-3}$mol L^{-1} than method [28], It is less expensive than methods [27, 28], besides it is characterized by wide linear range, low LOD and near Nernstian slope, 59.5mV/decade, than that in case of our recently published method [31]. The data are given in Table 6, thus proving that it is a good pinaverium-ion selective electrode for the pure and pharmaceutical preparations with high accuracy and precision.

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