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Flow Injection Potentiometric Determination of Pancuronium Bromide in Pharmaceutical Preparation and Urine Samples Using Modified Carbon Paste Electrodes

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Carbon paste electrodes for pancuronium bromide was prepared based on ion association complexes of pancuronium bromide with sodium tetraphenylborate (NaTPB) or ammonium reineckate using dibutyl phthalate as solvent mediator and tetradodecylammonium tetrakis-(4-chlorophenyl)borate (ETH 500) as lipophilic additive. The sensors showed a near-Nernstian slope of 28.1 mV concentration decade^{-1} at 25 °C within the concentration range 6.31×10^{-6}—1.00×10^{-2} M in case of pancuronium-tetraphenylborate electrode and 26.6 mV concentration decade^{-1} in the concentration range 5.66×10^{-5}—1.00×10^{-2} M in case of pancuronium-reineckate electrode. The sensors were successfully applied for the potentiometric determination of pancuronium bromide in pharmaceutical preparation and biological fluids in batch and flow injection conditions.

Key words pancuronium bromide; modified carbon paste electrode; flow injection analysis; potentiometry

Pancuronium bromide, 1,1′-(3α,17β-diacetoxy-5α-androstan-2β,16β-ylene)bis(1-methyl-piperidinium) dibromide [15500-66-0] is general anaesthesia used in surgery for muscle relaxation and as an aid to endotracheal intubation[1] (Chart 1). Several methods of determination of this drug have been reported in the literature, including high performance liquid chromatography (HPLC),[2—6] capillary gas chromatography using nitrogen sensitive detection,[7] liquid chromatography-mass spectrometry[8—12] spectrophotometric,[13—15] fluorimetric,[16,17] and mass spectrometric,[18] methods. A potentiometric ion selective electrode method based on pancuronium-tetraphenylborate and pancuronium-dipicrylaminate has been reported.[19,20]

The aim of this study is to describe the construction, performance characteristics of pancuronium modified carbon paste electrodes based on the pancuronium-tetraphenylborate (Panc-TPB) and pancuronium-reineckate as electroactive material and dibutyl phthalate as solvent mediator and the analytical applications of these sensors in determination of pancuronium bromide in pharmaceutical preparation and biological fluids in batch and flow injection conditions.

Experimental

Reagents and Materials All chemicals were of analytical grade. Double distilled water was used throughout all experiments. Pure grade pancuronium bromide and the pharmaceutical preparation Bromurex Ampoule (2 mg/ml) were provided by Sedico Pharmaceutical Co., 6 October City, Egypt. Sodium tetraphenylborate (NaTPB), ammonium reineckate, dioctyl sebacate (DOS), tricresyl phosphate (TCP), tetradodecylammonium tetrakis-(4-chlorophenyl)borate (ETH 500) were obtained from Fluka (U.S.A.), dibutyl phthalate (DBP) and dioctyl phthalate (DOP) from Merck (Germany). Graphite powder (1—2 micron) from Aldrich (U.S.A.).

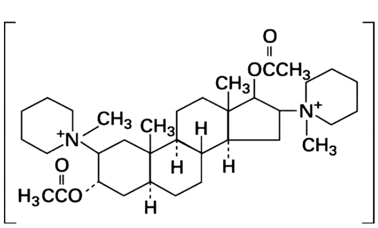

Chart 1

Apparatus Potentiometric and pH measurements were carried out using a Seibold G-103 digital pH/mV meter (Vienna, Austria). A Techne circulator thermostat Model C-100 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 wire was used as the internal reference electrode. The electrochemical system of the conventional electrode may be represented as follows: Panc-carbon paste electrode/test solution//KCl salt bridge//saturated calomel electrode.

The flow injection setup is that previously reported,[21,22] Fig. 1 represents the schematic diagram of the flow injection system used in the measurements.

Preparation of the Ion Pair The ion pair, Panc-TPB and Panc-reineckate were prepared by mixing 100 ml 10^{-2} M PancBr solution to 200 ml of 10^{-2} M of each 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 : 2 both in case of Panc(TPB)$_2$ and Panc(reineckate)$_2$ as confirmed by elemental analysis data done at microanalytical research laboratory in National Research Centre, Dokki, Cairo, Egypt. The percentage values found are 81.44, 7.90 and 2.40 and the calculated values are 82.22, 8.25 and 2.31 for C, H and N, respectively, in case of Panc(TPB)$_2$, while in case of Panc(reineckate)$_2$ the percentage values found are 41.34, 5.91, and 15.5 and the calculated are 41.46, 5.83, and 15.74 for C, H, and N, respectively.

Electrode Assembly Carbon paste was prepared by mixing the required amount of the ion pair, lipophilic additive with graphite powder (Aldrich, 1—2 micron) and dibutyl phthalate as a pasting liquid (ratio of graphite

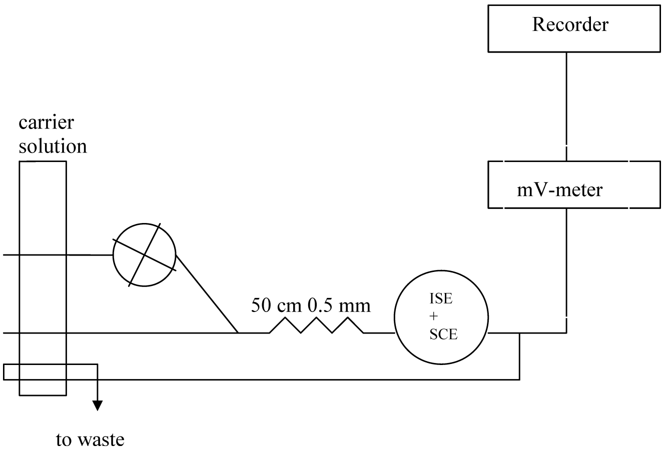

Fig. 1. Schematic Diagram of the Flow Injection System Used in the Measurements

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powder to pasting liquid was 1 : 1) in a mortar until it was uniformly wetted. The carbon paste electrode was prepared by successive packing of the carbon paste into the tip end of home made Teflon holder (2 mm) and electrical contact was achieved by stainless steel rod (2 mm) connecting the paste to the mV meter.

Potentiometric Determination of PancBr PancBr has been determined potentiometrically using the investigated electrodes by the standard addition method.[23]

Determination of PancBr in Bromurex Ampoule Two milliliters ampoule (2 mg PancBr/ml) were transferred to 10-ml measuring flask and completed to the mark by bidistilled water giving 5.459×10^{-5} M PancBr, then different amounts of this solution, 10 ml 0.1 M acetate buffer pH 4.7, (5 ml 1.0 M NaCl in case of Panc-TPB electrode) 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 PancBr by the standard addition method.

In FI, a series of solutions of different concentrations (2×10^{-5}—1×10^{-4} M) was prepared from the injections and the peak heights were measured, and then compared with those obtained by injecting a standard solutions of the same concentration prepared from pure PancBr.

Determination of PancBr in Spiked Urine Samples Different amounts of PancBr and 5 ml urine of a healthy person, 10 ml 0.1 M acetate buffer pH 4.7, (5 ml 1.0 M NaCl in case of Panc-TPB electrode) 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 PancBr by the standard addition method. In FI, the procedure was as previously described in case of Bromurex Ampoule.

Results and Discussion

Optimization of the Electrodes in Batch Conditions. Composition of Carbon Paste Electrode Four electrodes were prepared that contains the ion-pair (Panc-TPB or Panc-reineckate) in the ratio of 3, 5, 7, and 9% and the ratio of graphite to liquid mediators is 1 : 1. The presence of lipophilic anionic additives in cation selective electrodes not only diminishes the ohmic resistance and enhances the response behaviour and selectivity but also in case where the extraction capability is poor increase the sensitivity of the electrode,[24,25] and the ratio of lipophilic additives is about 0.5%.[26] In many of the new applications of ion selective electrodes a lipophilic additives consists of a lipophilic cation and lipophilic anion is used. 0.5% Tetradodecylammonium tetrakis-(4-chlorophenyl)borate as lipophilic additives was added (Table 1). The results showed that the carbon paste electrode with ion-pair ratio of 5% has the best performance characteristics (slope, 28.1 mV concentration decade^{-1} at 25 °C; usable concentration range, 6.31×10^{-6}—1.00×10^{-2} M PancBr, detection limit[27] 3.98×10^{-6} M, and response time $\leq$10 s) for Panc-TPB electrode, while, the characteristics are slope, 26.6 mV concentration decade^{-1} at 25 °C; usable concentration range, 5.66×10^{-5}—1.00×10^{-2} M PancBr, detection limit 2.42×10^{-5} M, and response time $\leq$10 s) for Panc-reineckate. The effect of plasticizers on the performance characteristics of ion selective electrodes are primarily due to their influence on migration, ion pair formation and diffusion coefficient.[28] The influence of plasticizer type on the performance characteristics of Panc sensors was investigated by using four plasticizers with different polarities (DBP, DOP, DOS, and TCP). The results reveal that DBP is the best of the plasticizers tested. Poor sensitivities for the electrodes plasticized by DOP, DOS and TCP are due to low distributions of electroactive complex Panc-TPB or Panc-reineckate in these solvents.[29] The electrodes using DBP as a plasticizer provided not only higher Nernstian slope but also a wider response range, lower detection limit and more stable potential readings. The response characteristics of the proposed electrodes were evaluated according to IUPAC recommendations (Table 2).[30]

Influence of pH and Effect of Electrolytes The influence of pH on the response of Panc sensors was checked for two concentrations of PancBr namely 1×10^{-3} and 1×10^{-4} M, by following the variation in potential with change in pH by spiking small volumes of hydrochloric acid and sodium hydroxide (each 0.1—1.0 M) in PancBr solutions. Potential *versus* pH profiles (Fig. 2) showed that the electrodes did not re-

Table 1. Composition of Carbon Paste (CP) Electrodes and the Slope of the Calibration Graphs at 25±1°C

Electrode	Composition (%, w/w)				Slope	S.D.
	Ion pair	ETH 500	Graphite	DBP	(mV decade^{-1})	
Panc-TPB						
I	3	0.5	48.25	48.25	26.3	0.438
II	5	0.5	47.25	47.25	28.1	1.463
III	7	0.5	46.25	46.25	26.8	0.550
IV	9	0.5	45.25	45.25	26.2	1.242
Panc-reineckate						
I	3	0.5	48.25	48.25	24.9	0.581
II	5	0.5	47.25	47.25	26.6	0.462
III	7	0.5	46.25	46.25	25.9	1.039
IV	9	0.5	45.25	45.25	25.2	0.564

Table 2. Response Characteristics of Panc Sensors

Parameter	Panc-TPB	Panc-reineckate
Slope (mV/decade)	28.1	26.6
Intercept (mV)	209.4	262.4
Linear range (M)	6.31×10^{-6}—1×10^{-2}	5.66×10^{-5}—1×10^{-2}
Detection limit (M)	3.98×10^{-6}	2.42×10^{-5}
Working pH range	2.15—7.40	2.36—7.45
Response time (s)	≤10	≤10

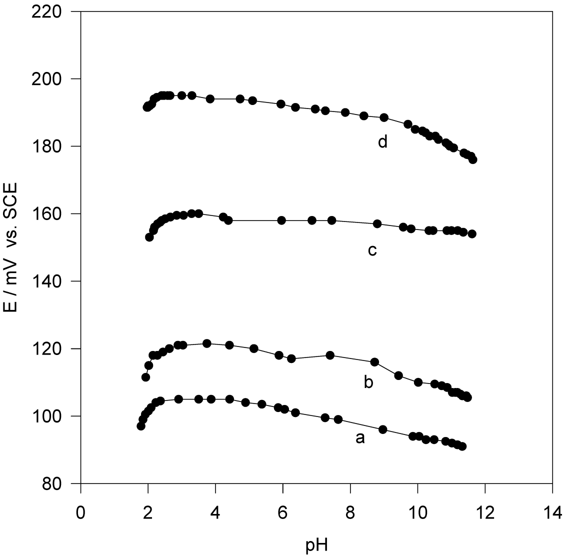

Fig. 2. Effect of the pH of the Test Solution Containing 1×10^{-4} and 1×10^{-3} M PancBr on the Potential Response in mV (E/mV) of the Panc Electrodes: (a and b) Panc-TPB and (c and d) Panc-Reineckate

sponse to pH changes in the ranges 2.15—7.40 and 2.36—7.45 for Panc-TPB and Panc-reineckate, respectively. The decrease in potential at lower pH may be related to interference of hydronium ion. The electrodes response was checked in bidistilled water, 0.04 M Britton Robinson buffer pH 4.7, 0.1 M acetate buffer pH 4.7 or 0.1 M citrate buffer pH 4.7, the best results were achieved in 0.1 M acetate buffer pH 4.7. The choice of a suitable ionic strength value at which the electrode exhibits the best response is also of prime importance in quantitative analyses. The potentials values of the electrodes at different electrolyte concentrations (0.01—1 M NaCl) have been determined in 0.1 M acetate buffer pH 4.7. The results indicate that in 0.1 M NaCl Panc-TPB electrode achieved the best response but in case of Panc-reineckate electrode the best response was achieved in 0.1 M acetate buffer without NaCl.

Life Time and Stability of the Electrodes The electrode life time was investigated by performing the calibration graphs periodically and calculating the response slope. The results indicate that the investigated electrodes exhibited stable potential reading in all the concentration ranges of the calibration graph, good stability in terms of slope in the linear domain of concentration and the electrodes can be used for about 6 and 2 months in case of Panc-reineckate electrode or Panc-TPB electrode without considerable decrease in the values of its slope.

The intra day and inter day (day-to-day) precision expressed as standard deviation were 0.541 and 0.874 ($n=4$) in case of Panc-TPB electrode or 0.174 and 0.778 ($n=4$) in case of Panc-reineckate.

Effect of Temperature To study the thermal stability of the electrodes, the calibration graphs are constructed at different temperatures, and the isothermal coefficients (dE/dt) of the electrodes[31] were found to be -0.0009 and -0.0002 V/°C for (Panc-TPB) and Panc-reineckate, respectively.

Optimization of the Electrodes Response in FI Conditions Flow injection analysis becomes a wide spread of methods which are characterized by its versatility, minium sample treatment prior to injection into the system, reduced time of analysis and low consumption of reagent compared to the manual procedure. FI parameters were optimized in order to obtain the best signal sensitivity. The dispersion coefficients were found to be 1.34 and 1.42 in case of Panc-TPB electrode and Panc-reineckate electrode, respectively, these limited dispersion coefficients aids the optimum sensitivity and fast response of the electrodes.[32] The effect of flow rate on the electrode response was assessed at different flow rate (4.15—30.00 ml/min), using 10^{-3} M PancBr solution with constant injection sample loope size of 75 μl. The residence time of the sample was inversely proportional to the flow rates.[33] It was found that, as the flow rate increases, the peak becomes higher and narrower until flow rates of 23.25 and 7.50 ml/min are reached in case of Panc-TPB and Panc-reineckate electrodes, respectively; the peaks obtained using more than these flow rates were nearly the same. These flow rates were used throughout this work providing the maximum peak height, shorter time to reach the base line and less consumption of the carrier solution. The influence of sample loope size on the response of the electrodes was studied by injecting samples of different volumes (4.7—500.0 μl) of 10^{-3} M PancBr at constant flow rate of 23.25 and 7.50 ml/min in case of Panc-TPB and Panc-reineckate electrodes, respectively. Sample loopes of size 150 and 75 μl in case of Panc-TPB and Panc-reineckate electrodes, respectively, were used throughout this work, giving maximum peak height, less consumption of reagents, and a short time to reach the base line.

Under these conditions, the performance characterstics are: slope, 29.2 or 38.0 mV concentration decade^{-1}, linear range, 5×10^{-5}—1×10^{-2} M and detection limit 2×10^{-5} or 3.98×10^{-5} M for Panc-TPB or Panc-reineckate electrodes, respectively. Figure 3 represents a typical recording and the calibration graph for Panc-TPB electrode as representative figure. The little increase in the background mV in Fig. 3a may be attributed to memory effect and it eliminated from the peak values before construction of Fig. 3b.

Selectivity of the Electrodes The influences of some inorganic cations, sugar, amino acids, vitamins and urea on the response of pancuronium sensors were investigated according to IUPC recommendations using the separate solution method,[34] or matched potential method (MPM)[35] in 0.1 M acetate buffer pH 4.7. The selectivity coefficient $K^{\text{pot}}_{\text{Panc},J^{z+}}$ measured by the separate solution method was calculated from Nicolsky Eisenman equation:

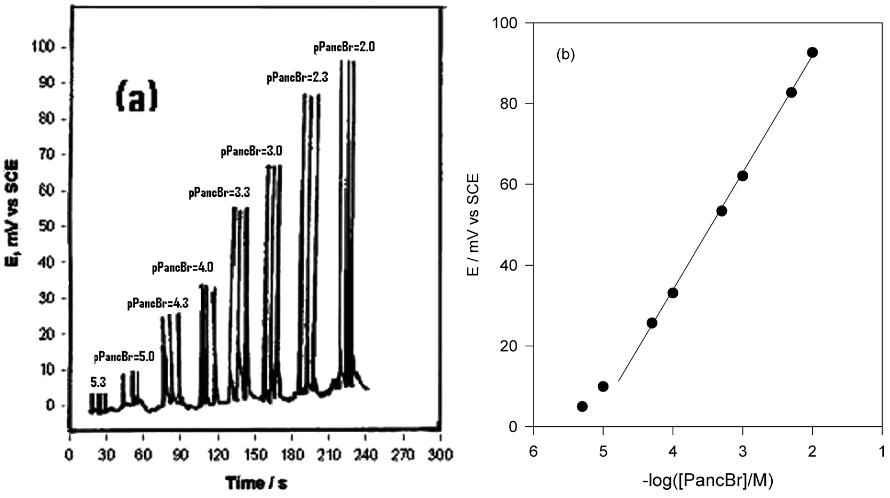

Fig. 3. Recording (a) and Their Corresponding Calibration Graph (b) for Panc-TPB Electrode under FI Conditions

$$\log K^{\mathrm{pot}}_{\mathrm{Panc},J^{z+}} = (E_2 - E_1)/S + \log[\mathrm{PanBr}] - \log[J^{z+}]^{1/z}$$

Where E_1 and E_2 are the electrode potential in a 1×10^{-3} M PancBr solution and interfering ions J^{z+}, respectively, and S is the slope of the calibration graph in mV concentration decade^{-1}. In FI conditions, a series of standard solutions of PancBr its concentration between 5×10^{-5} M and 1×10^{-2} M were prepared, their corresponding potentials were measured and used to determine the slope of the calibration graph. Solutions of 1×10^{-3} M of interfering ions were prepared, and their corresponding peak heights measured. The selectivity coefficient $K^{\mathrm{pot}}_{\mathrm{Panc},J^{z+}}$ were calculated using the above Nicolsky Eisenman equation. In cases of ions without charges the selectivity coefficients were determined by the matched potential method (MPM).[35)] In this method a known activity of pancuronium ion solution is added into a reference solution containing a fixed activity of pancuronium ion α_{Panc} ($\alpha'_{\mathrm{Panc}} - \alpha_{\mathrm{Panc}}$ is the change in activity), and the corresponding potential change (ΔE) is recorded, then a solution of an interfering ion is added to the reference solution until the same potential change (ΔE) is reached. The change in potential produced at the constant background of the primary ion must be the same in both cases.

$$K^{\mathrm{pot}}_{\mathrm{Panc},J^{z+}} = \frac{\alpha'_{\mathrm{Panc}} - \alpha_{\mathrm{Panc}}}{\alpha_J}$$

Where α_J is the activity of the added interferent.

The selectivity coefficients values, $-\log K^{\mathrm{pot}}_{\mathrm{Panc},J^{z+}}$, of the electrodes presented in Table 3 indicate that the prepared carbon paste electrodes are highly selective to pancuronium cation under both batch and flow injection conditions. In FI measurements, the sample remains in contact with the electrode for a short period of time, consequently, the selectivity coefficients must be different from that found in batch conditions. The inorganic cations do not interfere because of the difference in their mobility and permeability as compared to pancuronium 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 pancuronium cation.

Validity of the Proposed Methods, Robustness The robustness[36)] was examined by evaluating the effect of small changes in pH (4.4—5.0). The recovery values were not significantly affected by these variations and consequently the optimized procedure was reliable for assaying PancBr and the proposed methods could be considered robust.

Ruggedness Ruggedness[36)] was examined by applying the proposed procedures to assay PancBr using two different mV meter, Seibold G-103 digital pH/mV meter Vienna, Austria and a digital Schott Gerate pH/mV meter, Model CG820. The results obtained were reproducible, since there is no significant difference between the recovery and standard deviation values.

Analytical Applications In order to access the applicability of the proposed sensors, these sensors were applied for determination PancBr in bulk solutions, pharmaceutical preparation (Bromurex Ampoule) using standard addition method and flow injection analysis. The obtained average recovery and relative standard deviation values are summarized in (Table 4), the results were compared with the British pharmacopoeia spectrophotometric method.[37)] The results are in

Table 3. Selectivity Coefficients for the Panc Electrodes in Batch and FI Conditions

Interferent	$-\log k^{\mathrm{pot}}_{\mathrm{Panc},J^{z+}}$					
	Panc-TPB			Panc-reineckate		
	Batch		FI	Batch		FI
	SSM	MPM		SSM	MPM	
$\mathrm{Na^+}$	2.48	—	1.76	2.18	—	2.06
$\mathrm{K^+}$	2.28	—	1.90	2.00	—	2.30
$\mathrm{Ca^{2+}}$	3.74	—	2.83	3.64	—	3.31
$\mathrm{Mg^{2+}}$	4.11	—	2.78	3.79	—	3.17
$\mathrm{Zn^{2+}}$	4.10	—	2.76	3.69	—	3.00
$\mathrm{Co^{2+}}$	4.35	—	2.69	3.90	—	3.14
$\mathrm{NH_4^+}$	2.10	—	1.59	2.23	—	2.33
Vitamin $\mathrm{B_1}$	2.60	—	0.97	1.54	—	1.64
Vitamin $\mathrm{B_6}$	2.49	—	1.22	1.95	—	1.99
Glucose	—	2.28		—	2.49	
Fructose	—	2.52		—	2.68	
Lactose	—	2.53		—	2.77	
Maltose	—	2.32		—	2.52	
Glycine	—	2.53		—	2.78	
Alanine	—	2.49		—	2.59	
Urea	—	2.45		—	2.77	

Table 4. Determination of PancBr in Pure Form and Pharmaceutical Preparations by Applying Standard Addition Method and FI Technique

	Panc-TPB			Panc-reineckate		
	Taken (M)	Recovery (%)	RSD (%)	Taken (M)	Recovery (%)	RSD (%)
Standard addition						
Pure solution	1.00×10^{-5}	98.41	1.410	6×10^{-5}	100.54	1.180
	5.00×10^{-5}	99.28	1.485	8×10^{-5}	99.61	0.891
	1.00×10^{-4}	97.54	0.369	1×10^{-4}	99.16	0.585
Bromurex Ampoule						
Standard addition	1.00×10^{-5}	97.41	1.071	6×10^{-5}	97.24	1.713
	3.00×10^{-5}	100.75	0.095	8×10^{-5}	98.49	1.605
	4.97×10^{-5}	98.94	1.748	1×10^{-4}	97.46	2.000
Average		99.03	0.971		97.73	1.773
FI	2.00×10^{-5}	99.14	1.002	2×10^{-5}	97.34	0.629
	5.00×10^{-5}	100.00	1.69	5×10^{-5}	97.42	1.025
	1.00×10^{-4}	97.49	0.733	1×10^{-4}	97.43	1.444
Average		98.88	1.142		97.40	1.033

good agreement with the nominal value and with the average recovery (97.04±1.0) obtained from the official method. Statistical comparison of the accuracy and precision of the proposed methods with the British pharmacopoeia official method was performed using student's *t*-test and the Fisher–Snedecor (*F* test) at a 95% confidence level.[38] The *t*- and *F*-values did not exceed the theoretical values; there is no difference in accuracy or precision between the proposed and the reference official method. The British pharmacopoeia spectrophotometric official method need vexatious sample preparations and time and chemicals consuming method compared with the suggested proposed methods.

On intravenous injection pancuronium bromide is rapidly distributed into body tissues, about 80% may be bound to plasma proteins. A small proportion is metabolized in the liver to metabolites with weak neuromuscular blocking activity. It is largely excreted in urine as unchanged drug and metabolites, a small amount is excreted in bile. The plasma elimination half-life is about 2 h.[1]

PancBr is one of important target compound in some cases of medical malpractice and incidents such as suicide and murder. So obtaining the concentration of PancBr in urine samples collected from suicide is very important to legal medicine.[39]

The calibration graphs for the proposed investigated electrodes under the optimum conditions were constructed in absence and presence of urine samples (Fig. 4). The results indicate that the presence of urine not affect the response characteristics of proposed electrodes (slope, 27.0 mV concentration decade^{-1} at 25 °C within the concentration range 6.31×10^{-6}—1.00×10^{-2} M in case of pancuronium-tetraphenylborate electrode and 26.3 mV concentration decade^{-1} in the concentration range 5.66×10^{-5}—1.00×10^{-2} M in case of pancuronium-reineckate electrode.

Determination of PancBr in spiked urine samples was carried at three different levels of concentration (1×10^{-5}, 3×10^{-5} and 5×10^{-5} M) in batch condition using standard addition method and two concentration levels (1×10^{-5} and 1×10^{-4} M) in FI condition. The mean recovery and relative standard deviations were calculated and summarized in Table 5.

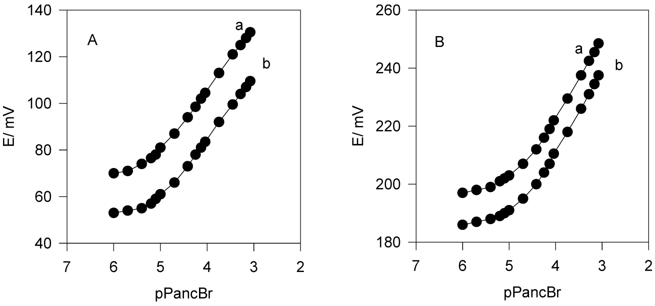

Fig. 4. Calibration Graphs of Panc-TPB Electrode (A) and Panc-Reineckate Electrode (B) in Absence of Urine Curve (a) and in Presence of Urine Curve (b)

Conclusion

The proposed pancuronium modified carbon paste electrodes based on pancuronium-tetraphenylborate and pancuronium-reineckate as electroactive material offers a simple, accurate, robust and selective tools for the determination of pancuronium bromide in bulk solution, pharmaceutical preparation and spiked urine samples in batch and FI conditions. Comparing the proposed electrodes with the previous published electrodes, the proposed electrodes has much longer life time than the previous published electrodes and these may be due to the presence of lipophilic additive. The novelty of this work is based on using Panc-reineckate carbon paste electrode as new electrode, using the proposed electrodes as potentiometric detectors in flow injection system for the determination of PancBr and long life time of these electrodes by addition of lipophilic additive.

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Table 5. Determination of Pancuronium Bromide in Spiked Urine Samples by Applying Standard Addition and FI Methods (n=4)

	Panc-TPB			Panc-reineckate		
	Taken (M)	Recovery (%)	RSD (%)	Taken (M)	Recovery (%)	RSD (%)
Standard addition method	1×10^{-5}	98.98	1.584	6×10^{-5}	100.02	0.657
	3×10^{-5}	100.07	1.361	8×10^{-5}	99.55	1.266
	5×10^{-5}	99.24	0.595	1×10^{-4}	99.49	1.699
FI method	1×10^{-5}	99.00	2.020	1×10^{-5}	100.00	1.739
	1×10^{-4}	98.93	0.723	1×10^{-4}	99.38	1.258

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