Rapid Determination of Gallamine Triethiodide (*Flaxedil*®) and Pancuronium Bromide (*Pavulon*®) in Pharmaceutical and Urine Matrices by Means of Modified-Carbon-Paste Ion-Selective Electrodes

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A new analytical method for the determination of gallamine triethiodide (*Flaxedil*®; **1**) and pancuronium bromide (*Pavulon*®; **2**), two muscle relaxants used in surgical operations and in pain relief, has been developed. Our approach relies on rapid, precise, and sensitive potentiometric sensors based on modified-carbon-paste ion-selective electrodes (CP-ISEs). Linear calibration graphs in the working ranges of *ca.* 4.5 – 892 and 7.3 – 733 μg/ml (in H_2O, pH 7.0, $T = 25°$) were established for **1** and **2**, respectively; and *Nernst* slopes corresponding to three- or two-electrons transfers, respectively, were obtained. The method works best in a pH range of 7 – 9. Average relative errors of 2.12 and 2.14%, with average standard deviations of 1.98 – 2.47 and 2.64 – 3.45, respectively, were obtained for urine samples of **1** and **2**. The corresponding relative errors for the pharmaceutical samples were 1.59 and 1.64%, with standard deviations of 0.54 – 1.34 and 0.52 – 1.67, respectively. Statistical *Student* and *F* tests were applied to the data, and satisfactory results were obtained.

1. Introduction. – The two drugs gallamine triethiodide (*Flaxedil*®; **1**) and pancuronium bromide (*Pavulon*®; **2**)[2]) are muscle relaxants used in surgical operations and for relief pain. These compounds possess only weak chromophores, which limits their detection by classical HPLC analysis based on UV and refractive-index measurements [1][2]. Reported HPLC methods based on mass-spectrometric and fluorescence detection with pre- or post-column derivatizations are costly and complicated. Therefore, reliable, accurate, and economical analytical methods are still needed for analysis. The aim of this investigation was to develop new, rapid, sensitive, and reliable carbon-paste ion-selective electrodes (CP-ISEs) for the determination of **1** and **2** in pharmaceutical and urine samples.

Gallamine triethiodide (**1**) and pancuronium bromide (**2**) are colorless, hygroscopic, crystalline powders freely soluble in H_2O and EtOH, and are typically 98 – 100% pure. They have to be freshly prepared and stored in air-tight containers protected from light. Almost 100% of intravenously administered gallamine (40 – 120 mg) is excreted unchanged in urine within 24 – 30 h [3]. In contrast, only *ca.* 25% of pancuronium bromide (40 – 100 μg/kg body weight) is excreted unchanged in urine, together with 15% of 3-hydroxy metabolites; and 10% are excreted in the bile [4].

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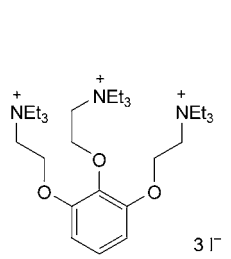
[2]) Systematic names: 2,2′,2″-[benzene-1,2,3-triyltris(oxy)]tris[*N*,*N*,*N*-triethylethanaminium] triiodide (**1**) and (2*β*,3*α*,5*α*,16*β*,17*β*)-3,17-bis(acetyloxy)-2,16-bis(1-methylpiperidinium-1-yl)androstane dibromide (**2**).



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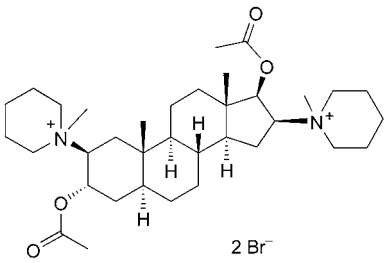

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Liquid-membrane ion-selective electrodes based on tetraphenylborate (TPB) and dipicrylaminate have been used for determining some muscle relaxants, with detection limits of 0.8–9.0 μM [5]. Potentiometric titration with $HClO_4$ is the method accredited in the European pharmacopoeia for determining gallamine triethiodide (**1**) and pancuronium bromide (**2**) [4][6]. A cathodic adsorptive stripping-voltammetric determination of trace amounts of **1** in phosphate media has also been reported [7].

Some quaternary ammonium compounds have been determined in urine by means of pyrolysis GC/MS and TLC, with recoveries of 58–98% [8]. They were also determined in serum by means of chemical-ionization mass spectrometry (CI-MS) in the concentration range of 1–500 ng/ml [9]. Gallamine triethiodide (**1**) in human and rat plasmas, muscle tissue, and microdialysate, with quantification limits of 1.0, 1.6, and 0.5 μg/ml, respectively, have been determined by HPLC and TLC [10–13].

On contrary to gallamine triethiodide (**1**), a literature search did not reveal the application of ion-selective electrodes for determining pancuronium bromide (**2**). This drug and its metabolites have been determined spectrofluorimetrically in human maternal and umbilical serums [14], as well as postmortem in blood and urine after suicide [15]. Analysis of pancuronium impurities by means of normal-phase HPLC has been reported [16]. A spectrophotometric method based on formation of an ion-pair complex between Bromophenol Blue, quinine, and **2** in a 2:2:1 ratio has been published [17]. Also, Rose Bengal has been used as a pairing agent for the fluorimetric determination of pancuronium in urine samples from a murder victim [18].

Quality control of pancuronium bromide (**2**) and its degradation products are typically made by HPLC [19]. Direct-inlet, electron-impact ionization mass spectrometry (EI-MS) was used for the determination of **2** extracted with CH_2Cl_2 from urine as its potassium iodate ion pair [20]. A method based on LC-MS for determining pancuronium bromide has also been reported [21].

2. Experimental. – 2.1. *General.* All chemicals were of anal. grade. Distilled H_2O from glass equipment was used throughout. Carbon powder (1–2 μm of grain size) was purchased from *Fluka*. Pure gallamine triethiodide (**1**) and pancuronium bromide (**2**) were purchased from *Sigma*. Dioctyl phthalate (DOP), dibutyl phthalate (DBP), tricresyl phosphate (TCP), and 2-nitrophenyl octyl ether (NPOE), used as plasticizers, were also purchased from *Sigma*. Ampoules of **1** (40 mg) and **2** (4 mg) in pyrogenic H_2O (2 ml each) were purchased from *Alexandria Pharmaceutical Industry* and *Novartis*, resp. Anal.-grade NH_4Cl, KCl, NaCl, sodium tetraphenylborate (NaTPB), urea, bromazepam, diazepam, K_2HPO_4, KH_2PO_4, and potassium tetrakis(4-chlorophenyl) borate were purchased from *Sigma*. Phosphate buffer (pH 7.0, $\mu = 0.444$) was prepared by mixing 390 ml of 0.1M NaH_2PO_4 with 610 ml of 0.1M Na_2HPO_4, adjusting the pH with a glass electrode.

2.2. *Stock Solns.* Stock solns. (0.5M) were freshly prepared each time by dissolving the drugs (44.575 g of **1** or 36.635 g of **2**) in distilled H_2O (50.0 ml) with continuous stirring. The resulting solns. were then made up to 100.0 ml final volume in a volumetric flask. Working solns. in the concentration range of $10^{-1}-10^{-7}$ M were prepared by accurate serial dilution. NaTPB (0.02 or 0.03M) solns. in distilled H_2O (50 ml) were standardized by potentiometric titration against 0.03M $AgNO_3$ using an Ag/AgS electrode *vs.* a double-junction saturated *Calomel* reference electrode. A series of KCl (32.0 mg/l), NaCl (145.0 mg/l), and standard solns. (10^{-4} M each) of NH_4Cl, urea, bromazepam, diazepam, **1**, and **2**, resp., were prepared in distilled H_2O (100 ml each).

2.3. *Ion-Pair Complexes.* To a stirred, 0.01M soln. of **1** or **2** in distilled H_2O (25 ml), a 0.02 or 0.03M soln. of NaTPB was added dropwise. The resulting precipitate was left overnight to settle down, filtered through a *G-4* sintered-glass crucible, and washed with distilled H_2O until no iodide or bromide ions were detected in the filtrate. Precipitated ion pairs were dried *in vacuo* for 48 h, and their compositions were confirmed by elemental and IR analyses.

2.4. *Electrodes.* A double junction *Calomel* electrode (Hg/Hg_2Cl_2), containing 3.0M KCl, was used as reference electrode. The working electrode was prepared as follows. Carbon paste was prepared by mixing accurately weighed (250.0 or 500.0 mg), highly pure carbon powder (1–2 μm) with the above ion-pair complexes (10–50 mg) and plasticizer (150 mg; DOP, TCP, DBP, or NPOE) in a battery dish. The mixture was thoroughly mixed, until uniformly wetted paste was obtained. The electrode was prepared by careful successive packing of the modified-carbon paste into the tip end of a *Teflon* holder (i.d. 2 mm). Electrical contact was achieved by a stainless-steel rod (2 mm) connecting the paste to the potentiometer (*Beckman 4500*). A fresh surface was obtained by gently pushing the stainless-steel screw forward and polishing the new carbon-paste surface with filter paper to obtain a shiny new surface. Fabricated electrodes were soaked in drug soln. (10^{-3} M) for 24 h before use. All potentiometric experiments were performed at $25\pm1°$, and pH measurements were carried out with a combined pH glass electrode (*HANNA HI-8417*), with a sensitivity of ±0.05 pH units.

2.5. *Procedures.* 2.5.1. *Calibration Graphs.* Standard solns. of **1** or **2** ($10^{-1}-10^{-7}$ M) were prepared by transferring accurate aliquots into a 50-ml thermostatted cell, to which phosphate buffer (pH 7; 5 ml) was added. The electrochemical potentials (in mV) of these solns. were directly measured with the corresponding CP-ISE at 25°. Calibration graphs were constructed by plotting recorded potentials *vs.* logarithmic concentration values. Slopes of the resulting calibration curves were calculated and used for subsequent determination of drug ions in soln.

2.5.2. *Drug Determination in Pharmaceutical Samples.* A 2-ml ampoule of *Flaxedil*® (**1**; 40 mg) was evacuated into a 50-ml volumetric flask, which was filled up to the mark with distilled H_2O. Successive aliquots (1–7 ml) were transferred to the measuring cell, to which phosphate buffer (pH 7; 5 ml) was added, before diluted with H_2O to a total volume of 50 ml. This resulted in solns of **1** with concentrations equivalent to 17.83–89.15 μg/ml. Similarly, a 2-ml ampoule of *Pavulon*® (**2**; 4 mg) was evacuated into a 25-ml volumetric flask, which was filled up to the mark with H_2O. Aliquots of 2.5–32.5 ml were transferred to the measuring cell. Addition of phosphate buffer (pH 7; 5 ml) followed by dilution with H_2O resulted in solns. of **2** having concentrations equivalent to 8.00–104.01 μg/ml. The resulting solns. were once directly measured and once potentiometrically titrated *vs.* 10^{-3} M NaTPB using the corresponding CP-ISE at 25°. Concentrations of gallamine or pancuronium were calculated from the previously constructed calibration graphs.

2.5.3. *Drug Determination in Urine Samples.* To 25-ml portions of human urine, aliquots (1–7 ml) of a 10^{-3} M standard soln of **1** or **2** were transferred to 50-ml measuring flasks. Phosphate buffer (pH 7; 5 ml) was added, and the solns. were made up to the mark with distilled H_2O, giving rise to concentrations of 17.83–124.81 and 14.65–102.58 μg/ml of **1** and **2**, resp.. The resulting solns. were once directly measured and once potentiometrically titrated *vs.* 10^{-3} M NaTPB at 25°. Recorded potentials were corrected for dilution, and plotted *vs.* volume of added NaTPB.

2.5.4. *Potentiometric Titration of Pure Samples.* Accurate aliquots (1–8 ml) of the 10^{-3} M, freshly prepared standard solns of **1** or **2** were transferred into a 50-ml thermostatted cell, phosphate buffer (pH 7; 5 ml) was added, and the volume was made up to 50 ml with distilled H_2O. The resulting solns. were potentiometrically titrated *vs.* 10^{-3} M standard NaTPB at 25°, using the corresponding CP-ISE. Recorded potentials were corrected for dilution, and plotted *vs.* the volume of added NaTPB.

2.5.5. *Selectivity Coefficients.* Separate drug solns. with primary ion (i) and interfering secondary ion (j), having activities a_i and a_j, resp., were prepared. The corresponding electrochemical potentials E_i and E_j were measured. Selectivity coefficients K were calculated by means of *Eqn. 1*, where Z is the ion charge and S the slope of the primary-ion calibration curve:

$$\log K_{ij} = [(E_j - E_i)/S)] + \log a_i - (Z_i/Z_j) \log a_j \quad (1)$$

3. Results and Discussion. – Graphite consists of stratified giant molecules formed of condensed benzene-like rings [22]. Although, the mechanism of conduction in carbon paste is still unclear, response mechanisms similar to liquid-membrane electrodes composed of plasticizers and ion exchangers have been assumed [23]. Charge transfer through the carbon paste can be attributed to electrons transferred between drug ions diffused within the carbon paste coupled to electronic charge transfer at the Fe surface. Therefore, the activity of drug ions diffusing within the paste is related to the potential of the electrode. Paste composition, thus, should affect electrode behavior, signal-to-noise ratio, and response time. The electrochemical potential developed at the solution/paste interface is dependent on the analyte activity in the drug solution analyzed.

Tetraphenylborate (TPB^-) within the paste is selective for the cationic drug ions (D^+). Consequently, cationic conduction could be attributed to the exchange $D^+Br^- + Na^+TPB^- \rightarrow D^+TPB^- + NaBr$, with the drug–TPB ion pair diffusing into the carbon paste. Three amino groups are assumed to be reduced in case of gallamine triethiodide (**1**), whereas two are susceptible to reduction in case of pancuronium bromide (**2**). These functional groups represent the physical basis of the three- or two-electron transfers responsible for potentiometric activity. Introduction of lipophilic substances such as dioctyl phthalate (DOP), dibutyl phthalate (DBP), tricresyl phosphate (TCP), and 2-nitrophenyl octyl ether (NPOE) as binding materials in the carbon paste may also help in prohibiting H_2O transport, thus, changing the activities of the electro-active species [24].

3.1. *Electrode Composition.* Elemental analyses showed that TPB forms ion pairs with gallamine triethiodide (**1**) and pancuronium bromide (**2**) in ratios of 1:3 and 1:2, respectively. Modified-carbon-paste electrodes were prepared using different amounts of ion pairs and different plasticizers (*Table 1*). Pastes with ion pairs of 2, 5, and 10 weight-% relative to the carbon powder were prepared and tested. The paste with 10%

Table 1. *Performance Characteristics of Carbon-Modified Ion-selective Electrodes for the Determination of* **1** *and* **2** *as a Function of Electrode Composition and Binding Material*. Standard deviations were calculated based on at least three calibrations. The working pH range was 7–9 in all cases.

BM[a])	Composition[b])	Slope [mV/decade]	R	Working range [μg/ml]	Detection limit [μg/ml]
Compound **1**:					
DOP	500 : 0.15 : 10	54.09 ± 2.8	0.88	8.92 – 892.00	8.92
DOP	500 : 0.15 : 25	49.72 ± 3.74	0.86	8.92 – 892.00	4.45
DOP	250 : 0.15 : 25	14.85 ± 0.38	0.98	4.46 – 892.00	0.89
DBP	250 : 0.15 : 25	19.30 ± 0.52	0.99	4.46 – 892.00	0.89
TCP	250 : 0.15 : 25	16.28 ± 1.16	0.99	8.92 – 892.00	0.89
NPOE	250 : 0.15 : 25	12.31 ± 1.08	0.96	8.92 – 892.00	8.92
Compound **2**:					
DOP	250 : 0.15 : 25	35.92 ± 4.77	0.97	7.33 – 732.68	7.32
DBP	250 : 0.15 : 25	31.15 ± 2.32	0.98	7.33 – 732.68	0.73
TCP	250 : 0.15 : 25	42.33 ± 3.59	0.99	7.33 – 732.68	0.73

[a]) Binding material: DOP = dioctyl phthalate, DBP = dibutyl phthalate, TCP = tricresyl phosphate, NPOE = 2-nitrophenyl octyl ether. [b]) Ratio of carbon powder [mg] to binding material [ml] to ion pair [mg]

ion-pair content showed the best *Nernst* slopes and correlation coefficients. The plasticizers DOP, DBP, TCP, and NPOE were tested as binding materials. CP-ISEs based on DBP showed the best slopes over wide concentration ranges. Thus, modified CP-ISEs with 5% of ion pairs and DBP as binding material were selected for all further investigations.

3.2. *Electrode Performance.* CP-ISEs based on drug – TPB ion pairs as electro-active materials, with DOP, DBP, TCP or NOPE as binding materials, were investigated. *Table 1* shows dynamic ranges calculated using regression analysis. It also shows the detection limits based on ion activities at which measured potentials tended to deviate from the linear part of the calibration by more than $18/z$ mV, where z is the number of electrons transferred.

In *Fig. 1*, the electrochemical calibration graphs of **1** and **2** are shown as a function of concentration. Cationic slopes of 14.85 ± 0.38, 19.30 ± 0.52, 16.28 ± 1.16, and 12.31 ± 1.08 mV/decade were obtained for **1** with DOP, DBP, TCP, and NOPE, respectively. Slopes of 35.92 ± 4.77, 31.15 ± 2.32, and 42.33 ± 3.59 mV/decade were obtained for **2** with DOP, DBP, and TCP, respectively (*Table 1*). Since TCP, DOP, and NOPE plasticizers showed relatively large deviations from a *Nernst* slope, they were excluded

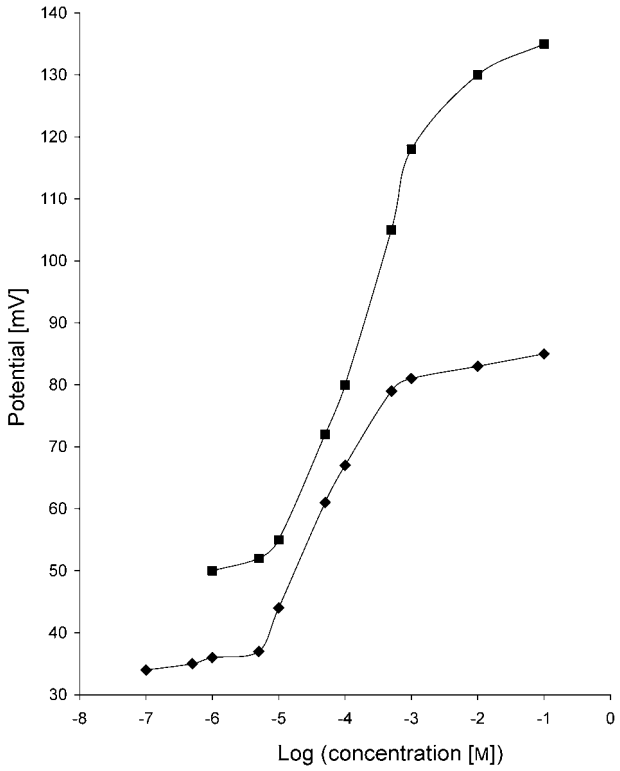

Fig. 1. *Calibration graphs for gallamine triethiodide* (**1**; ◆) *and pancuronium bromide* (**2**; ■) *determined by means of modified-carbon-paste ion-selective electrodes.* The paste was prepared with 10 weight-% ion pair and DBP as binding material (see *Experimental*); $T = 25°$.

for further investigation. Subsequent determinations were carried out with DBP. Linear dynamic ranges, extended over 2 to 3 orders of magnitude, were obtained. Detection limits reached down to 0.89 and 0.73 μg/ml gallamine (**1**) and pancuronium (**2**), respectively, indicating good performance characteristics.

Reproducibility was tested by repeating each measurement at least three times. Insignificant differences in potential readings were obtained when an electrode was immersed in standard solutions with the same concentration from different batches, or when several electrodes with the same composition were immersed in the same standard solution. Good reproducibility is indicated by the low standard deviations given in *Table 1*.

3.3. *Effect of pH.* In *Fig. 2*, the effect of solution pH on electrode potential is shown for 10^{-3} and 10^{-4} M drug solutions. An insignificant effect was observed on the electrode potentials within a pH range of *ca.* 7–9. At pH <7, deviation from linearity can be attributed to increased response to H^+ ions. At pH >9, gallamine (**1**) forms the basic form, which results in lower ionic activity and causes the electrode potential to decrease [25]. The observed increase in the potential of pancuronium (**2**) solutions at pH

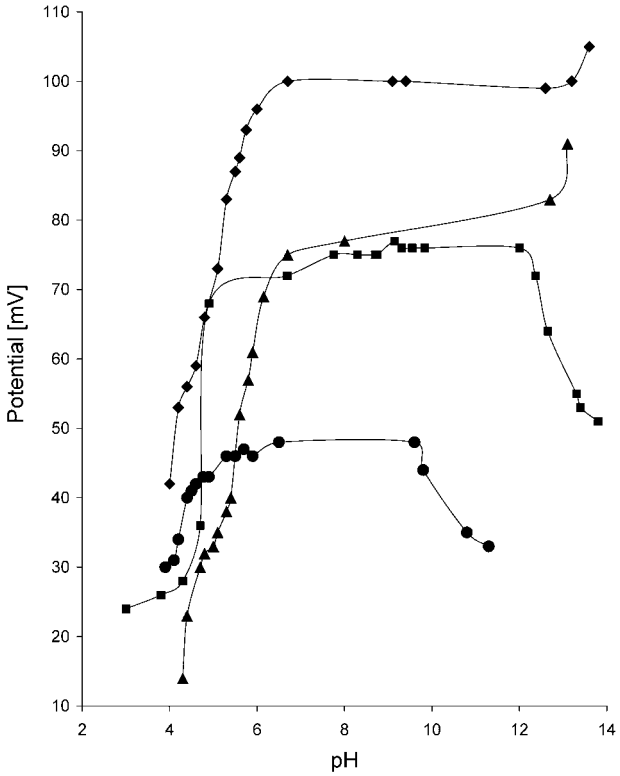

Fig. 2. *Effect of pH on the electrochemical potential measured with modified-carbon-paste ion-selective electrodes.* Legend: ■ and ● represent 10^{-3} and 10^{-4} M solutions of **1**, resp.; ▲ and ◆ stand for 10^{-3} and 10^{-4} M solutions of **2**, resp.; $T=25°$.

>13 might be caused by drug hydrolysis. Thus, the optimum pH range for drug determination is 7–9.

3.4. *Life Span and Response Time.* Long-term stability of electrode potential is an important parameter in practical applications, and a large potential drift is a major drawback. Potential stabilities of gallamine (**1**) and pancuronium (**2**) CP-ISEs were monitored over one month by measuring their potentials at 10^{-3} and 10^{-4} M concentrations. Electrodes were considered no longer suitable for measurements when the potential differences exceeded ± 5 mV. The potentials of all electrodes were shown to be significantly stable and remain operational during this time span (1 month). Moreover, an average electrode response time of 10 s was recorded for the above solutions.

3.5. *Selectivity.* Interferences caused by organic and inorganic ions were tested by means of the 'separate-solution method' [26]. Interfering standard solutions of KCl, NaCl, NH_4Cl, urea, bromazepam, diazepam, **1**, and **2** were tested (*Table 2*). Selectivity coefficients K in the range of 0.006–0.230 and 0.010–0.440 were obtained for **1** and **2**, respectively. Urea showed relatively low interference coefficients of 0.001–0.002, which should allow one to use the electrodes in urine samples. The electrodes exhibited fair selectivity in detecting mixtures of **1** in the presence of **2**, and *vice versa*, with K values of 0.23 (**1**) and 0.44 (**2**).

Table 2. *Selectivity Coefficients* (K_{ij}) *for Selected Interfering Ions on Gallamine Triethiodide* (**1**) *and Pancuronium Bromide* (**2**) *Detection with Carbon-Paste Ion-Selective Electrodes.* The K values were determined according to *Eqn. 1* (see *Experimental*).

Interfering ion	Concentration	$K_{i,j}$	
		1	**2**
Na^+	145 ppm	0.016	0.051
K^+	32 ppm	0.056	0.031
NH_4^+	10^{-4} M	0.060	0.140
Urea	10^{-4} M	0.010	0.020
Bromazepam	10^{-4} M	0.004	0.010
Diazepam	10^{-4} M	0.055	0.021
1 or **2**[a])	10^{-4} M	0.230	0.440

[a]) Compound **1** in tests with **2**, and *vice versa*.

3.6. *Gallamine Triethiodide and Pancuronium Bromide Assays.* Modified CP-ISEs with a content of 10% ion-pair compound and DBP as binding material were used for assaying compounds **1** and **2** (*Table 3*). Direct potentiometric determination using the calibration-curve method gave mean recoveries of 100.91 and 99.46%, respectively. Least squares for four calibration graphs gave confidence limits for the slope and intercept at a confidence level of 95%. The regression equations for **1** and **2** were $Y = (19.30 \pm 0.52) \cdot X + (140.75 \pm 2.08)$ and $Y = (31.15 \pm 2.32) \cdot X + (208.72 \pm 7.69)$, respectively, where X is the average logarithmic molar concentration and Y the average potential (in mV). Excellent correlation coefficients of $R \geq 0.98$ were obtained (*Fig. 1*). These equations can, thus, be readily used for the determination of the corresponding drugs in pharmaceutical and urine samples.

Table 3. *Analyses of Compounds* **1** *and* **2** *in Pure and Pharmaceutical Samples by Means of Direct-Batch* vs. *Potentiometric-Titration Methods*

	Direct batch				Potentiometric titration				
Taken [μg/ml]	Found [μg/ml]	Recovery [%]	SD (CV)[a])	t[b])	Found [μg/ml]	Recovery [%]	SD (CV)[a])	t[b])	F[c])
1 (pure)									
17.83	18.20	102.08	2.05 (11.26)	0.53	18.72	104.99	2.06 (11.00)	0.75	2.00
53.49	54.79	102.43	1.82 (2.32)	1.24	56.45	105.53	2.20 (3.90)	2.33	1.46
89.15	90.12	101.09	1.53 (1.70)	1.10	89.69	100.61	1.98 (2.21)	0.47	1.67
124.81	124.03	99.38	1.45 (1.16)	0.93	132.65	106.28	2.41 (1.82)	5.63	2.76
2 (pure)									
14.65	14.12	96.38	1.98 (14.02)	0.46	15.78	107.76	2.06 (13.05)	0.95	1.08
43.96	45.07	102.53	1.15 (2.62)	1.67	45.16	102.73	1.08 (2.39)	1.92	1.13
73.27	73.63	100.49	0.85 (1.16)	0.73	73.71	100.60	1.12 (1.52)	0.68	1.74
102.58	103.93	101.32	1.20 (1.15)	1.95	105.51	102.86	1.45 (1.37)	3.50	1.46
1 (from *Flaxedil*®)[d])									
17.83	17.89	100.34	0.91 (5.09)	0.11	20.68	115.98	0.67 (3.24)	7.37	1.84
53.49	54.64	102.15	0.54 (0.99)	3.69	55.55	103.85	0.52 (0.94)	6.86	1.08
89.15	91.45	102.58	1.34 (1.50)	2.97	90.93	102.00	1.45 (1.60)	2.13	1.17
2 (from *Pavulon*®)[d])									
14.40	14.45	100.35	0.88 (7.07)	0.10	14.96	103.89	0.60 (4.20)	1.62	2.15
20.80	21.41	102.93	0.52 (2.82)	2.03	20.88	100.38	0.62 (3.07)	0.22	1.42
104.01	104.38	100.36	1.67 (0.67)	0.45	107.71	103.56	1.54 (1.50)	4.16	1.18

[a]) Standard deviations (SD) and variance coefficients (CV) based on the average of at least three determinations. [b]) *Student* test values: $t = [(\bar{X} - X)\sqrt{N}]/\mathrm{SD}$. [c]) F Values for direct *vs.* potentiometric results: $F = (\mathrm{SD}_1)^2/(\mathrm{SD}_2)^2$. [d]) *Flaxedil*® and *Pavulon*® ampoules contained 40 and 4 mg of **1** and **2**, resp., in 2 ml of H_2O each.

In *Fig. 3*, potentiometric titrations of **1** and **2** are shown for a 10^{-3} M solution of NaTPB as titrant. Symmetrical titration curves with well-defined potential jumps above 100 mV were obtained, indicating high sensitivity. Recoveries of 100.60–110.61%, with standard deviations in the range 1.08–2.41 indicate good precisions (*Table 3*).

3.7. *Analysis of Pharmaceutical Samples.* Direct determinations and potentiometric titrations of gallamine (**1**) in *Flaxedil*®, and of pancuronium (**2**) in *Pavulon*® ampoules are shown in *Table 3*. Standard deviations in the range of 0.54–1.34 and 0.52–0.88, with average recoveries of 101.23 and 101.64%, respectively, were obtained. Regression equations ('taken' *vs.* 'found') could be represented by $Y = 1.03 \cdot X - 0.51$ and $0.985 \cdot \mathrm{X} - 3.03$ for gallamine (**1**), and by $Y = 1.01 \cdot X + 0.11$ and $1.001 \cdot X + 2.04$ for pancuronium (**2**), where X is the average 'taken' (= true value) and Y is the average 'found' (in μg/ml). Correlation coefficients of 1.0 were obtained in all cases. Statistical treatment using the *Student* test gave t values of 0.11–3.69 and 0.10–2.03 for **1** and **2**, respectively. This indicates insignificant differences between the measured and the real values at a confidence level of 95%.

Fig. 4 shows a successful application of our CP-ISEs as end-point-indicator electrodes for potentiometric titrations of gallamine (**1**) and pancuronium (**2**) in the commercial ampoules, when using 10^{-3} M NaTPB as titrant. Symmetric titration curves

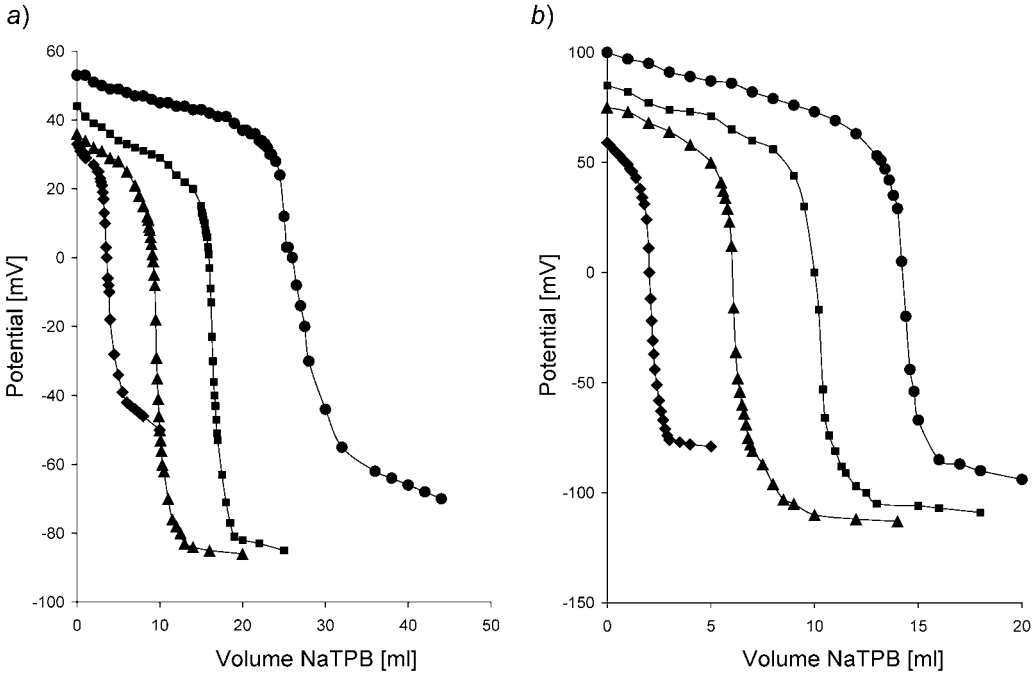

Fig. 3. *Potentiometric titration curves for* a) *pure gallamine triethiodide* (**1**) *and* b) *pure pancuronium bromide* (**2**) *as a function of the amount* [ml] *of sodium tetraphenylborate* (NaTPB; 10^{-3} M stock solution). Legend: ◆ = 1 ml, ▲ = 3 ml, ■ = 5 ml, and ● = 7 ml (10^{-3} M drug solutions); $T = 25°$.

with well-defined potential jumps above 100 mV, indicating high sensitivity, were obtained. Recovery of 102–116%, with standard deviations in the range 0.60–3.07, confirm the suitability of the prepared electrode for the determination of the two drugs in pharmaceutical samples (*Table 3*). Applying the '*F* test' for comparing the direct and potentiometric titration methods gave mean *F* values smaller than the critical ones, indicating insignificant difference in precisions between both methods (confidence level of 95%; *Table 3*).

3.8. *Analysis of Urine Samples.* Spiked urine samples containing different amounts of gallamine (**1**) and pancuronium (**2**) were analyzed by the direct-batch method (*Table 4*). Standard deviations in the range of 1.98–3.46 and 0.89–3.45, and average recoveries of 95.91–106.69% were obtained, respectively. 'Taken' *vs.* 'found' regression equations of $Y = 0.98 \cdot X - 0.36$ and $Y = 1.02 \cdot X + 0.01$, with correlation coefficients of 1.0, were deduced for **1** and **2**, respectively. *Student* test values *t* in the range of 0.00–2.40 were obtained, indicating insignificant differences between measured and real concentrations (confidence level of 95%; *Table 4*).

The potentiometric titration curves of the drug samples in urine, using 10^{-3} M NaTPB, are shown in *Fig. 5*. Potential jumps above 60 mV indicate the high relative sensitivity of our method. Estimating the difference in precisions between direct and potentiometric titration methods at a confidence level of 95% gave lower *F* values than the critical ones (*Table 4*).

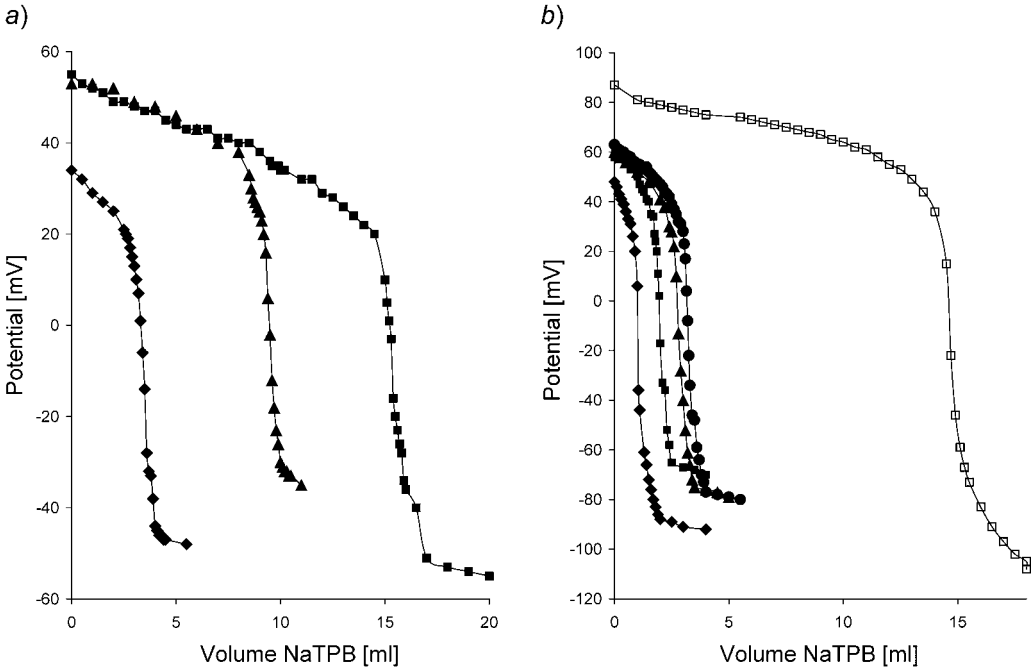

Fig. 4. *Potentiometric titration curves measured with modified-carbon-paste ion-selective electrodes for* a) *gallamine triethiodide* (**1**) *and* b) *pancuronium bromide* (**2**) *taken from pharmaceutical ampoules.* Legend: ◆ = 1.12 ml, ▲ 3.34 ml, and ■ = 5.57 ml of *Flaxedil*® (**1**) solution (9.0×10^{-4} M in H_2O); ◆ = 2.5 ml, ■ = 4.5 ml, ▲ = 6.5 ml, ● = 7 ml, and □ = 32.5 ml of *Pavulon*® (**2**) solution (2.18×10^{-4} M in H_2O). Titrant: sodium tetraphenylborate (10^{-3} M); $T = 25°$.

Table 4. *Analyses of Compounds* **1** *and* **2** *in Urine Samples by Means of Direct-Batch vs. Potentiometric-Titration Methods*

	Direct batch				Potentiometric titration				
Taken [μg/ml]	Found [μg/ml]	Recovery [%]	SD (CV)[a])	t[b])	Found [μg/ml]	Recovery [%]	SD (CV)[a])	t[b])	F[c])
Compound **1**									
17.83	17.10	95.91	2.24 (13.10)	0.56	17.83	100.00	2.40 (13.46)	0.00	1.15
35.66	34.45	96.61	1.98 (5.75)	1.06	34.77	97.51	0.73 (2.10)	2.11	7.36
53.49	52.87	98.84	2.35 (4.44)	0.46	52.00	97.21	1.36 (2.62)	1.90	2.99
89.15	87.15	97.76	2.47 (2.83)	1.40	86.59	98.25	2.23 (2.58)	1.99	1.23
Compound **2**									
14.65	15.18	103.62	2.77 (18.25)	0.33	15.41	105.19	0.89 (5.78)	1.48	9.69
43.95	44.19	100.54	2.64 (5.97)	0.16	46.89	106.69	2.12 (4.52)	2.40	1.55
73.25	74.80	102.12	3.45 (4.61)	0.78	76.20	104.03	3.20 (4.20)	1.60	1.16

[a]) Standard deviations (SD) and variance coefficients (CV) based on the average of at least three determinations. [b]) *Student* test values: $t = [(\bar{X} - X))\sqrt{N}]/\mathrm{SD}$. [c]) F Values for direct *vs.* potentiometric results: $F = (\mathrm{SD}_1)^2/(\mathrm{SD2})^2$.

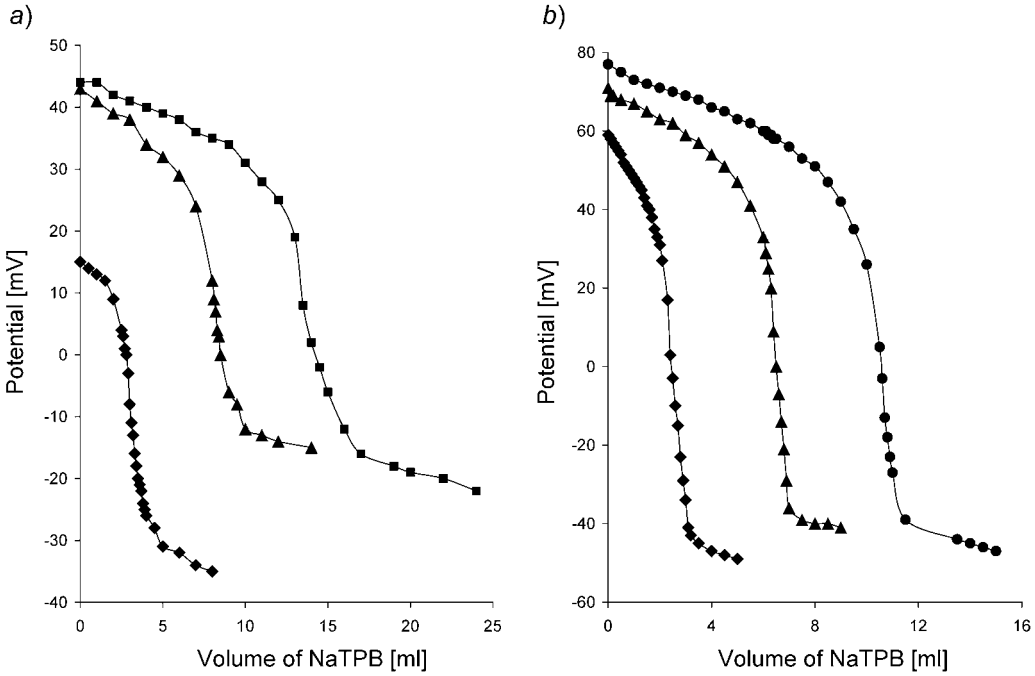

Fig. 5. *Potentiometric titration curves measured with modified-carbon-paste ion-selective electrodes for different amounts of spiked* **1** (a) *and* **2** (b) *in urine.* Legend: ◆ = 1 ml, ▲ = 3 ml, and ■ = 5 ml of **1** (10^{-3} M) in urine; ◆ = 1 ml, ▲ = 3 ml, and ● = 5 ml of **2** (10^{-3} M) in urine. Titrant: sodium tetraphenylborate (10^{-3} M); $T = 25°$.

4. Conclusions. – We have developed novel carbon-paste ion-selective electrodes (CP-ISEs) for the simple, accurate, inexpensive, and sensitive determination of gallamine triethiodide (**1**) and pancuronium bromide (**2**), both in pharmaceutical and urine samples. The electrodes showed good performance characteristics and were stable during at least one month. The possibility of using these electrodes in media where symmetrical ISEs are difficult to operate make our electrodes privileged for analytical applications and quality control. Detection limits of 0.89 and 0.73 μg/ml towards **1** and **2**, respectively, are far less than reached with pharmacopeial methods, and lie below the excreted amounts of these drugs in urine. Interference from urine matrix or electrode fouling, as occurring by proteins in liquid-membrane and coated-wire ion-selective electrodes, were not seriously observed. Thus, our CP-ISEs can be successively used as indicator electrodes for the potentiometric titrations of these drugs.

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