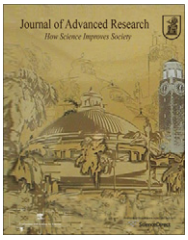
Journal of Advanced Research (2011) 2, 25–34

Cairo University

Journal of Advanced Research



ORIGINAL ARTICLE

Using PVC ion-selective electrodes for the potentiometric flow injection analysis of distigmine in its pharmaceutical formulation and biological fluids

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Received 11 January 2010; revised 1 June 2010; accepted 21 June 2010
Available online 13 October 2010

KEYWORDS

Ion-selective electrode;
PVC-membrane;
Distigmine determination;
Urine;
Flow injection analysis;
Dissolution profile;
Potentiometry

Abstract The construction and electrochemical response characteristics of poly(vinylchloride) (PVC) membrane selective electrodes for the determination of distigmine (Ds) are described. The sensing membrane comprised an ion-pair based on distigmine phosphomolybdate (Ds-PM), distigmine phosphotungstate (Ds-PT), distigmine silicomolybdate (Ds-SM), distigmine silicotungstate (Ds-ST), distigmine tetraphenylborate (Ds-TPB), and distigmine reineckate (Ds-Rein) in a plasticized PVC matrix with dioctylphthalate (DOP). The influence of membrane composition on the electrodes' response was studied. The electrodes showed a fast, stable and Nernstian response over a wide distigmine concentration range 5.0×10^{-7}–1×10^{-2} mol L^{-1} with a slope of $\sim$30.5 ± 1.0 mV dec^{-1}. The response is independent of the pH of test solution within the range 3.8–10.5. The life span of the electrodes extends to at least 2 months without any considerable divergence in potential and has a fast response time of < 15 s. The electrodes showed good selectivity towards distigmine with respect to large numbers of ions in batch and FIA systems. The electrodes have been applied to the determination of distigmine in pure solution, pharmaceutical compound and human urine. The dissolution profile for Ubretid tablets (5 mg/tablet) was studied.

Introduction

Distigmine is one of several drugs that have been used to treat myasthenia gravis. Distigmine, therefore, improves contraction of muscle. Distigmine [1] increases the amount of acetylcholine that is available to stimulate the remaining receptors, therefore enhancing muscle contraction. Distigmine bromide, [15876-67-2], also known as bispyridostigmine bromide or hexamarium bromide, is 3,3′-[*N,N*′-hexamethylenebis(methylcarbamoyloxy)]bis-(1-methylpyridinium bromide) (Fig. 1), and is a parasympathomimetics quaternary ammonium compound for the treatment of myasthenia gravis that is a reversible inhibitor

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Peer review under responsibility of Cairo University.
doi:10.1016/j.jare.2010.08.007

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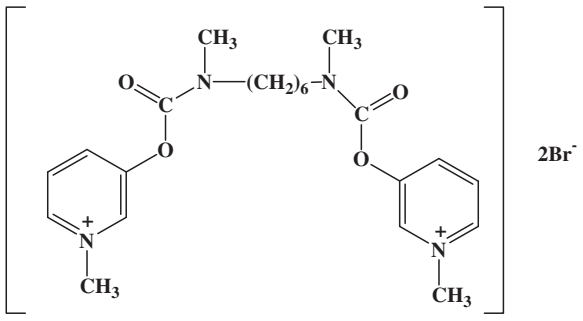

Fig. 1 The chemical structure of distigmine bromide.

of cholinesterase activity with actions similar to those of neostigmine but more prolonged. It is also used in conditions where the muscle in the intestine wall has become paralysed (paralytic ileus). The reported methods for the determination of distigmine are few, including chromatographic [2–6], mass spectrometry [7] and spectrophotometric [8]. In recent years, potentiometric membrane ion-selective electrodes (ISEs) have been extensively used in pharmaceutical and biological analysis [9–18]. This is mainly due to their simple design, low cost, adequate selectivity, good accuracy, wide concentration range and applicability to colored and turbid solutions.

A thorough literature survey revealed no methods involving selective electrodes for the determination of distigmine. Therefore, the aim of this work is to develop an ion-selective electrode for distigmine determination and its application for determining this drug in pure solution, pharmaceutical preparations and human urine. Flow injection analysis and dissolution profile of Ubretid tablets (5 mg/tablet) were also considered.

Experimental

Reagents and materials

All reagents used were chemically pure grade. Doubly distilled water was used throughout all experiments. Distigmine bromide and its pharmaceutical preparation (Ubretid tablets, 5 mg/tablet) were provided by the Arab Drug Company, Cairo-A.R.E. under License of NYCOMED-Austria. Poly(vinyl chloride) (PVC) of high molecular mass and tetrahydrofuran (THF) were obtained from the Aldrich chemical company.

The stock solution was prepared to contain 0.01 mol L^{-1} $DsBr_2$ and was standardized spectrophotometrically by measuring the absorbance of its solutions at 270 nm and 242 nm [19]. Dioctyl phthalate (DOP), tricresyl phosphate (TCP), tributyl phosphate (TBP) and dibutyl phthalate (DBP) were used as the most suitable plasticizer in PVC membranes received from Aldrich. Acetonitrile and dimethylformamide (DMF) were obtained from Aldrich. Corn oil, sodium hydroxide, and silver nitrate were from local sources. Aqueous solutions (0.01 mol L^{-1}) of silicotungstic acid (STA), silicomolybdic acid (SMA), phosphotungestic acid (PTA), phosphomolybdic acid (PMA), sodium tetraphenylborate (NaTPB) and ammonium reineckate (AmmRein) were prepared. The exact concentrations of these solutions were determined by the appropriate recommended methods [20–22].

Apparatus

The potentiometric measurements in batch were carried out with a Jenway 3510 digital pH/mV meter. A techno circulator thermostat Model C-100 (Cambridge-England) was used to control the temperature of the test solution. A WTW-packed saturated calomel electrode (SCE) was used as an external reference electrode.

A single-stream FIA system was used. It is composed of a four-channel peristaltic pump (Ismatec, ISM 827) (Zurich, Switzerland) and an injection valve model 5020 with an exchangeable sample loop from Rheodyne (Cotati CA, USA). The electrodes were connected to a WTW micro-processor pH/ion-meter pMx 2000 (Weilheim, Germany) and interfaced to a strip chart recorder model BD 111 from Kipp and Zonne (Deflt, Netherlands). A wall-jet, thin-layer and flow-through cell can be applied to this system [23].

The dissolution was carried out according to the USP XXX [24] method with the paddle apparatus [25]. The standard equipment used for this purpose is the Pharmatest model "SR8Plus", CA, USA, Hanson Research, serial number "73-100-116" (CHATSWORTH) [26,27], and the UV–Visible spectrophotometer (Japan).

Preparation of ion-exchangers

The ion-exchangers, distigmine phosphomolybdate (Ds_3-PM_2), distigmine phosphotungstate (Ds_3-PT_2), distigmine silicomolybdate (Ds-SM_2), distigmine silicotungstate (Ds-ST_2), distigmine tetraphenylborate (Ds-TPB_2) and distigmine reineckate (Ds-$Rein_2$), were prepared by adding 50 ml of 10^{-2} mol L^{-1} distigmine bromide ($DsBr_2$) solution to 100 ml of each of the following: 0.0033 mol L^{-1} of PMA, 0.0033 mol L^{-1} of PTA, 0.0025 mol L^{-1} of SMA, 0.0025 mol L^{-1} of STA, 0.01 mol L^{-1} of NaTPB, and 0.01 mol L^{-1} of AmmRein. The formed precipitates were filtered off, washed thoroughly with distilled water till bromide free (as tested by acidic solution of $AgNO_3$), then dried at room temperature and ground to fine powder. The formation and purity of the ion-pairs and ion-associates and the chemical compositions of the precipitates were checked by elemental analysis for carbon, hydrogen and nitrogen at the Microanalytical Center, Faculty of Science, Cairo University. The results are given in Table 1.

Construction and preparation of membrane electrodes

Membranes of different compositions were prepared. The percentages of each ion-exchanger were changed to cover the ranges of 0.5–5.0%, Ds_3PM_2, Ds_3PT_2, $DsSM_2$, $DsST_2$, $DsTPB_2$, and $DsRein_2$.

The membranes of optimum composition were prepared by dissolving the required amounts of PVC and DOP in 5 ml THF. It was found that distigmine tetraphenylborate, and distigmine reineckate are soluble in THF while distigmine phosphomolybdate, distigmine phosphotungstate, distigmine silicomolybdate and distigmine silicotungstate are soluble in THF–acetonitrile or THF–dimethylformamide mixtures. The calculated amount of ion-exchanger was dissolved in acetonitrile/THF mixture (2:7) and mixed with the PVC/DOP solution in Petri-dish (7.0 cm diameter). The total weight of

Table 1 Elemental analyses of the ion-associates.

Ion-associate	Color	Tentative formulae		C%	H%	N%
Ds_3PM_2	G. yellow	$[C_{22}H_{32}N_4O_4]_3[PMo_{12}O_{40}]_2$	Found	17.07	6.05	3.66
			(Calc.)	16.86	5.98	3.43
Ds_3PT_2	White	$[C_{22}H_{32}N_4O_4]_3[PW_{12}O_{40}]_2$	Found	11.40	2.97	2.39
			(Calc.)	11.31	2.80	2.39
$DsSM_2$	Buff	$[C_{22}H_{32}N_4O_4]_4[SiMo_{12}O_{40}]_2$	Found	20.56	6.05	4.21
			(Calc.)	20.08	6.34	4.22
$DsST_2$	White	$[C_{22}H_{32}N_4O_4]_4[SiW_{12}O_{40}]_2$	Found	14.25	2.06	3.16
			(Calc.)	14.23	2.20	3.02
$DsTPB_2$	White	$[C_{22}H_{32}N_4O_4][C_{24}H_{20}B]_2$	Found	79.03	7.30	5.27
			(Calc.)	79.66	6.98	5.30
$DsRein_2$	Magenta	$[C_{22}H_{32}N_4O_4][Cr(NH_3)_2(SCN)_4]_2$	Found	33.53	5.87	20.66
			(Calc.)	33.91	5.51	21.01

constituents in each batch was fixed at 0.35 g. To obtain homogeneous and uniform thickness, the membranes were left to dry freely in air for 24 h. In each case, a disk of the membrane with a 12 mm diameter was punched from the large membrane and glued to the polished end of a 2 cm long PVC plastic cap attached to one end of a 10 cm glass tube homemade electrode body. The electrodes were filled with a solution of 10^{-1} mol L^{-1} with respect to KCl and 10^{-3} mol L^{-1} with respect to distigmine solution and preconditioned by soaking in 10^{-3} mol L^{-1} of the drug solution. The electrochemical system is represented as follows: Ag/AgCl//inner solution/membrane/test solution/SCE.

Construction of the calibration graphs

For batch measurements, suitable increments of standard drug solutions were added to 50 ml doubly distilled water so as to cover the concentration range 1.0×10^{-7}–1.0×10^{-2} mol L^{-1}. The sensor and the reference electrodes were immersed in the solution and the emf values were recorded at 25 ± 1 °C; after each addition the values were plotted versus the negative logarithmic value of the drug concentration (pDs). For FIA measurements, a series of freshly prepared solutions of the drug covering the range 1.0×10^{-6}–1.0×10^{-1} mol L^{-1} were injected into the flow stream and the corresponding peak heights were recorded and used to draw the calibration graphs.

Response time of the ion-selective electrodes

The response time is the time which elapses between the instant when an ion-selective electrode and a reference electrode (ISE cell) are brought into contact with a sample solution (or at which the activity of the ion of interest in a solution is changed) and the instant at which the emf/time slope ($\Delta E/\Delta t$) becomes equal to a limiting value selected on the basis of the experimental conditions and/or some requirements concerning the accuracy. The response time of the investigated electrodes was calculated on the basis of this definition.

Selectivity of the electrodes

According to the MPM [28], the selectivity coefficients of different interfering ions for the studied electrodes, to a reference solution containing (a_{Ds}) is added an amount of the drug to give a final concentration of (a'_{Ds}); the shift in potential change (Δ) is thus measured. To a reference solution containing the same concentration (a_{Ds}), a certain amount of interference ion that causes the same (Δ) value is thus determined (a_j)

$$K^{MPM}_{Ds,j} = \frac{\Delta a_{Ds}}{a_j} = \frac{a'_{Ds} - a_{Ds}}{a_j}$$

In FI conditions the separate solution method was applied [29]. It requires two potential measurements: the first potential is measured in a solution containing a known concentration of the drug and the second potential is measured in a solution containing the same concentration of interfering ion. The two potential values were measured at the tops of the peaks for the same concentration of the drug and the interferent.

Potentiometric determination of $DsBr_2$

In batch measurements, the standard addition method was applied [30], in which a known incremental change is made through the addition of standard solution to the sample. This was achieved by adding known volumes of standard drug solution to 50 ml water containing different amounts of the investigated drug in its pure state, pharmaceutical preparation (Ubretid tablets), and in urine samples spiked with known amounts of the drugs. The change in mV reading was recorded for each increment and used to calculate the concentration of the drug in sample solution using the following equation [30]:

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

where C_x is the concentration to be determined, V_x is the volume of the original sample solution, V_s and C_s are respectively the volume and concentration of the standard solution added to the sample to be analyzed, Δ is the change in potential after the addition of certain volume of standard solution, and S is the slope of the calibration graph.

Potentiometric titrations

An aliquot of the investigated compound containing 2.88–57.63 mg $DsBr_2$ was transferred into a 100 ml titration cell

and diluted to 50 ml by distilled water; the resulting solutions were titrated against 0.0033 mol L^{-1} PMA, 0.0033 mol L^{-1} PTA, 0.0025 mol L^{-1} SMA, 0.0025 mol L^{-1} STA, 0.01 mol L^{-1} NaTPB, 0.01 mol L^{-1} AmmRein, and 0.01 mol L^{-1} picric acid, using the corresponding electrode(s). The end-points were determined from the conventional S-shaped curves, the first and second derivative plots.

Determination of $DsBr_2$ in spiked urine

For urine analysis, different quantities of the drug and 5 ml urine were transferred to a 100 ml volumetric flask, completed to the mark with doubly distilled water and a small volume (0.1–2.0 ml) 0.01 mol L^{-1} HCl was added to give solutions of pH ranging from 4 to 5 and concentrations from 1.0×10^{-6} to 2.8×10^{-4} mol L^{-1} drug. These solutions were subjected to the standard addition method for drug determination.

Determination of $DsBr_2$ using FI system

In FIA, samples of different concentrations of Ubretid solutions were injected into the optimized FIA system. The peak heights were measured and compared to those obtained from injecting standard solutions of pure drug.

Dissolution

One tablet of Ubretid (5 mg/tablet) was placed in the vessel of 16-tablet dissolution instrument and the dissolution medium (500 ml of 0.01 mol L^{-1} HCl) was maintained at 37 ± 0.5 °C. It should be noted that the expected maximum concentration after complete dissolution of the tablet will be 1.74×10^{-5} mol L^{-1}. The vessel was rotated at 50 rpm [31]. At appropriate time intervals, the potential values were recorded using the distigmine sensor in conjunction with the saturated calomel electrode (SCE), reference electrode, and the amount of distigmine released was calculated from the calibration graph. For the spectrophotometric measurements, 5.0 ml aliquots of the dissolution solution were withdrawn, filtered, diluted with 0.01 mol L^{-1} HCl, and the absorbance measured at 270 nm [32]. A calibration graph was used for drug release calculation.

Results and discussion

Influence of membrane composition in batch conditions

Several membranes of a varying nature and ratio of ion-exchanger/PVC/plasticizer were prepared for the systematic investigation of each membrane composition. Experimental trials proved that a certain percentage of each ion-exchanger was optimum, indicated by the Nernstian behavior of the electrode. However, further increase of the ion-exchangers over this percentage resulted in a diminished response slope of the electrode, most probably due to some inhomogenities and possible saturation of the membrane [33]. Results are given in Table 2 and Fig. 2.

Effect of solvent mediators on the PVC membranes

The influence of the plasticizer type and its quantity on the characteristics of the studied sensors was investigated by using five plasticizers with different polarities including: DBP, DOP, TCP, TBP and corn oil. Different plasticizer/PVC (w/w) ratios were studied: the 1:1 plasticizer/PVC ratio produced maximum sensitivity for all of the plasticizers. The electrodes containing DOP generally showed better potentiometric responses, i.e. sensitivity and linearity range of the calibration plots [34–36].

Response time of the electrodes

The response time is defined as the time between the addition of analyte to the sample solution and the time when a

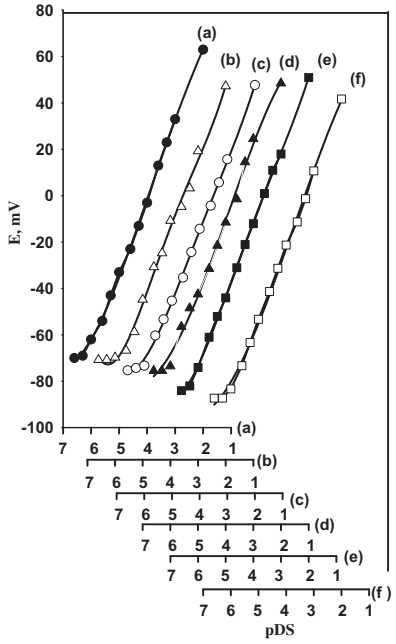

Fig. 2 Calibration graphs for Ds-PM (a), Ds-PT (b), Ds-SM (c), Ds-ST (d), Ds-TPB (e), and Ds-Rein (f) at optimum membrane composition.

Table 2 Optimum membrane composition and response characteristics of the Ds-electrodes.

Electrodes	Composition (%) (I.P%–PVC–DOP)	Slope (mV/decade)	Linearity range (M)	Limit of detection (M)	Working pH range	Response time (s)	Life span (days)
Ds-PM	3.0–48.5–48.50	31.0 ± 0.7	5.0×10^{-7}–1.0×10^{-2}	4.0×10^{-7}	3.8–10.5	⩽10–12	70
Ds-PT	1.0–49.5–49.50	29.4 ± 0.9	7.9×10^{-7}–1.0×10^{-2}	7.1×10^{-7}	3.8–10.5	⩽10–15	63
Ds-SM	0.5–49.75–49.75	30.5 ± 1.0	6.3×10^{-7}–1.0×10^{-2}	5.0×10^{-7}	3.8–10.5	⩽12–15	63
Ds-ST	0.5–49.75–49.75	28.1 ± 0.5	7.5×10^{-7}–1.0×10^{-2}	6.3×10^{-7}	3.8–10.5	⩽12–15	56
Ds-TPB	3.0–48.5–48.50	33.2 ± 1.0	4.0×10^{-7}–1.0×10^{-2}	3.5×10^{-7}	3.8–10.5	⩽10–12	77
Ds-Rein	3.0–48.5–48.50	30.5 ± 1.3	1.0×10^{-6}–1.0×10^{-2}	8.9×10^{-7}	3.8–10.5	⩽12–15	63

steady-state potential with less than 0.1 mV/min change has been achieved. The dynamic response time [37] of each electrode was tested by measuring the time required to achieve a steady-state potential (within ±1 mV) after successive immersions of the electrode in a series of drug solutions, each having a 10-fold increase in concentration from 1.0×10^{-6} to 1.0×10^{-2} mol L^{-1}. The electrodes yielded steady potential within 10–15 s. The potential readings stayed constant, to within ±1 mV, for at least 10 min. This is most probably due to the fast exchange kinetics of association–dissociation of distigmine ion with the ionophores at the solution–membrane interface. The potential–time plot for the response of the Ds-PM electrode is shown in Fig. 3.

Influence of pH

The effect of pH on the electrode potential at various distigmine concentrations in the range 1.0×10^{-5}–1×10^{-3} mol L^{-1} was studied. The pH was varied by adding HCl or NaOH and the results are shown in Fig. 4. As can be seen, the electrode potential was independent of pH in the range 3.8–10.5 for all the distigmine concentrations assayed, and in this range the electrodes can be safely used for distigmine determination. The slight change in potential readings at pH values lower than the previously mentioned ranges is attributed to interference of hydronium ions, while at pH higher than the given ranges the potential readings decrease gradually, which can be related to the deprotonation of the drug molecules.

Selectivity

The selectivity behavior is obviously one of the most important characteristics of an ion-selective electrode, determining whether a reliable measurement can be obtained by the electrode proposed. Thus, the potential response was investigated in the presence of various interfering foreign cations using the matched potential method (MPM). The MPM is a recommended procedure which avoids the limitations of the corresponding methods based on the Nicolsky–Eisenman equation for the determination of potentiometric selectivity coefficients.

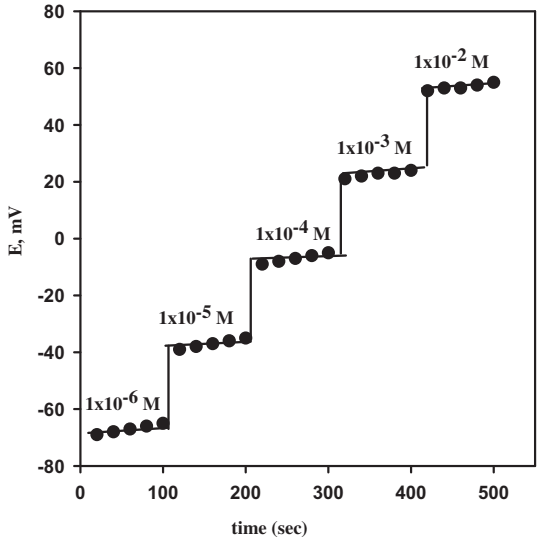

Fig. 3 Potential–time plot for Ds-PM electrode.

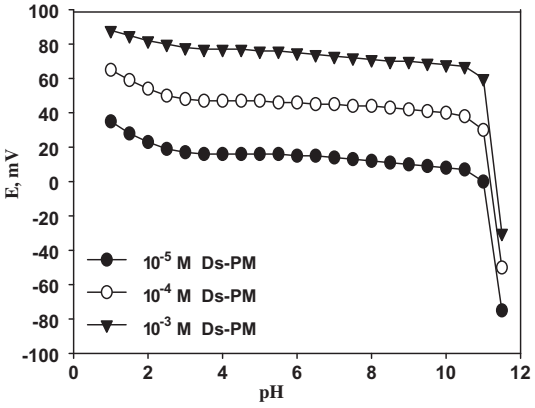

Fig. 4 Influence of pH at different distigmine concentrations on emf values using Ds-PM electrode.

These limitations include non-Nernstian behaviors of interfering ions and inequality of charges of any primary interfering ion. The inorganic cations do not interfere because of differences in ionic size, mobility and permeability.

In FI conditions, the values of selectivity coefficients were calculated based on potential values measured at the tops of the peaks for the same concentrations of the drug and the interferent according to the separate solution method [29].

The resulting values are listed in Table 3. As is evident, most of the interfering ions show low values of selectivity coefficient, indicating negligible interference in the performance of the membrane sensor assembly. Comparing the selectivity coefficients' values obtained for the investigated electrode both in batch and FI conditions (see Table 3), it is apparent that there are some differences between the values obtained in both cases for each interfering ion. This may be attributed to the different methods applied in determining the selectivity coefficient values in both batch and FI techniques, i.e. the matched potential and separate solution methods respectively [38–40]. This is interpreted by difference in times of interaction of interferents with the sensor in comparison to the main sensed ion; also the interference process is highly dependent on the rate of diffusion and the exchange reaction of the interfering ion [41].

Effect of temperature

To study the thermal stability of electrodes, calibration graphs (electrode potential, E_{elec} versus pDs) were constructed at different test solution temperatures covering the range of 25–50 °C. Plots of ($E^{\circ}_{elec.}$) versus (t_{-25}) for each electrode gave a straight line. The slope of the line was taken as the thermal coefficient of the electrode. The isothermal coefficient (dE_{elect}/dt) of each electrode was calculated [42] and found to be ~ -0.0001 V °C^{-1} and (dE_{cell}/dt) equals ~ 0.0005 V °C^{-1}. The small values of $(dE^{\circ}/dt)_{cell}$ and $(dE^{\circ}/dt)_{elec.}$ listed in Table 4 reveal the high thermal stability of the studied electrodes within the investigated temperature range and show no deviation from the theoretical Nernstian behavior.

Optimization of the electrodes' response in FIA conditions

Dispersion coefficient

Dispersion coefficient (D) is one of the most important factors to be taken into consideration in constructing a FIA system

Table 3 Selectivity coefficient values ($-\log K^{pot}_{Ds,J}z+$) for Ds-electrodes.

Interferent	PM	PT	SM	ST	TPB	Rein	PM (FIA)
Na^+	3.81	3.21	3.23	3.15	3.23	3.11	2.6
K^+	3.55	3.39	3.76	3.89	3.71	3.22	2.7
Ca^{2+}	4.23	3.65	3.21	3.65	3.21	3.32	2.9
Mg^{2+}	4.60	4.25	4.20	4.25	4.20	4.00	2.8
NH_4^+	3.80	3.61	3.23	4.01	4.23	4.23	2.2
Ba^{2+}	4.74	4.33	4.11	4.33	4.11	4.12	3.1
Mn^{2+}	4.33	4.24	4.27	4.24	4.27	4.23	3.0
Cu^{2+}	3.94	3.64	3.57	3.78	3.82	3.89	3.3
Cd^{2+}	4.63	4.33	4.24	4.33	4.24	4.11	3.1
Pb^{2+}	4.22	4.62	3.24	4.34	4.07	4.36	3.3
Sr^{2+}	4.35	4.28	3.23	4.28	4.23	4.36	3.3
Cr^{3+}	4.74	4.23	4.36	4.23	4.36	4.28	3.8
Al^{3+}	4.60	4.28	4.23	4.15	4.23	4.14	–
Glucose	4.44	4.23	4.39	4.23	4.39	4.28	–
Fructose	4.94	4.11	4.00	4.11	4.00	4.11	–
Maltose	4.78	4.08	4.21	4.08	4.21	4.31	–
Lactose	4.68	4.33	4.22	4.33	4.22	4.32	–
Urea	3.88	3.12	3.32	3.12	3.32	3.19	–
Vit. C	4.41	4.39	4.19	4.39	4.19	4.31	–
Thiamine HCl	2.87	3.98	3.72	3.98	3.72	3.97	2.79
Pyridoxine HCl	4.25	4.05	4.33	4.05	4.33	4.12	3.31
Folic acid	3.17	3.10	3.21	3.25	3.35	3.41	–
Citric acid	2.76	2.77	2.68	2.87	2.92	3.21	–
Theronine	4.61	4.11	4.21	4.11	4.21	4.34	–
L-Valine	4.33	4.34	4.08	4.34	4.08	4.17	–
L-Lysine	4.81	4.11	4.37	4.11	4.37	4.36	–
Asparagine	4.56	4.32	4.25	4.32	4.25	4.23	–
L-Arginine	4.45	4.11	4.10	4.20	4.24	4.33	–
DL-Serine	4.77	3.76	3.93	3.76	3.93	4.15	–
L-Proline	4.63	4.17	4.13	4.19	4.22	4.25	–
L-Cysteine	4.58	4.28	4.21	4.31	4.52	4.68	–
Alanine	4.43	4.32	4.10	4.32	4.10	4.09	–
Glutamic acid	4.88	4.48	4.43	4.54	4.64	4.71	–
Glycine	4.63	4.29	4.22	4.30	4.36	4.39	–

Table 4 The thermal coefficient values of cells and electrodes.

Electrodes	$(dE°/dt)_{cell}$	$(dE°/dt)_{elec.}$	Electrodes	$(dE°/dt)_{cell}$	$(dE°/dt)_{elec.}$
Ds-PM	3.4×10^{-4}	-1.4×10^{-4}	Ds-ST	5.2×10^{-4}	-2.2×10^{-4}
Ds-SM	4.0×10^{-4}	-3.0×10^{-4}	Ds-TPB	3.9×10^{-4}	-2.7×10^{-4}
Ds-PT	4.6×10^{-4}	-2.6×10^{-4}	Ds-Rein	5.5×10^{-4}	-1.6×10^{-4}

because it shows how much the original sample solution is diluted on its way towards the sensor and how much time has elapsed between the sample injection and the readout. The dispersion coefficient (D), defined as the ratio of concentrations of sample material before and after the dispersion process has taken place, can either be limited (D = 1–3), medium (D = 3–10) or large (D > 10) [43].

The dispersion coefficient, determined by measuring the ratio between the peak height obtained at steady-state conditions (where the sample acts as carrier stream) and at the state of maximum peak height, maximum dispersion (where the sample is injected in carrier stream), was found to be 1.21. This value is affected by many parameters such as sample volume, flow rate and channel geometry.

Carrier composition

The composition of the carrier should be as similar as possible to that of the sample; this is highly advantageous for baseline stability, response time and wash characteristics [44,45]. To stabilize the baseline, the carrier stream was made by using bi-distilled water as a carrier stream with respect to the analyzed drug. The use of other carrier solutions led to a decrease in the peak heights and to the higher consumption of reagents.

Injection volume

The influence of the injection volume on the performance of the detector response was assessed by proceeding to intercalation of volumes (20.0, 37.5, 75.0, 150.0, 340.0 and 500.0 μL) of the drug standard 10^{-3} mol L^{-1} solution, fixing the flow rate at

12.50 ml/min. A progressive increase in the intensity of the analytical signals was verified [46] by using the Ds-PM electrode as an example, and a sample loop of size 150 µL was used throughout this work as the most suitable.

Flow rate

The dependence of the peak heights and the time taken to recover the baseline on the flow rate was studied; the response of the electrodes under investigation, using 10^{-3} mol L^{-1} solution of the respective drug, was studied at different rates (4.15, 5.35, 7.50, 9.70, 12.50, 17.85, 23.25, 25.00 and 27.00 ml min^{-1}). Using a constant injection volume, the residence time of the sample is inversely proportional to the flow rate [47]. Therefore, low flow rate would seem likely to produce a steady-state signal but will also lead to increased response time due to increased residence time of the sample at the active electrode surface. It was found that, as the flow rate increased, the peaks become higher and narrower until the optimum flow rate is reached, where the peaks obtained above which are nearly the same.

A calibration curve was constructed for the optimized flow injection system based on the peak heights, which follow the expected Nernstian behavior, Fig. 5.

Analytical applications

The new investigated electrodes have been applied and were found to be useful in the potentiometric determination of $DsBr_2$ in tablets by standard addition method or potentiometric titration. In contrast to potentiometry, the potentiometric titration technique usually offers the advantages of high accuracy and precision, albeit at the cost of increased titrant consumption. A further advantage is that the potential break at the titration end-point must be well-defined, but the slope of the sensing electrode response neither needs to be reproducible nor Nernstian, and the actual potential value at the end-point is of secondary interest. The method for distigmine ion (Ds^{2+}) titration is based on the decrease of (Ds^{2+}) concentration by precipitation with PMA, PTA, STA, SMA, NaTPB, Amm-Rein or picric acid standard solution. The titration process was carried out manually in aqueous solution containing 9.9×10^{-5}–2.0×10^{-3} mol L^{-1} $DsBr_2$ with average recoveries of 98.50–100.9% and relative standard deviations of 0.23–1.41% for five measurements. The sudden emf change near the end-points amounts to approximately 84–112 mV in the case of titrating 1.9×10^{-5} mol L^{-1} $DsBr_2$ using Ds-PM electrode and increases gradually as the titrated amount of $DsBr_2$ increases, reaching 195–230 mV in case of titrating 2.0×10^{-3} mol L^{-1} $DsBr_2$ using the same electrode. Corresponding titration curves are shown in Fig. 6. The mean recovery values in the determination of tablet samples are shown in Table 5, and range from 98.5% to 101.0% with small relative standard deviations (RSD) values ranging from 0.36% to 1.55%. The standard additions method was proved to be successful for the determination of the investigated drug in its pure solutions. From the results shown in Table 5, it is clear that the obtained mean recovery values of the amounts taken of pure drug samples ranged from 98.5% to 101.3% with small RSD values 0.15–0.94%.

Also, the standard addition method was applied for determination of $DsBr_2$ in Ubretid tablet (5 mg/tablet). The results in Table 5 show that the percentage recovery for determination of tablet samples ranged from 99.5% to 101.7% with small RSD values (0.21–0.54%). The new distigmine-selective electrodes were satisfactorily applied to the determination of distigmine in human urine. In this application, urine samples were spiked with a known amount of drug to give concentration ranges that match the normal clinically relevant levels. Then, the samples were analyzed potentiometrically using the developed selective electrodes for assaying the drug. The standard addition technique was applied to overcome the matrix effects in these samples. The mean recovery values of the spiked amount of drug in urine samples (see Table 5) ranged

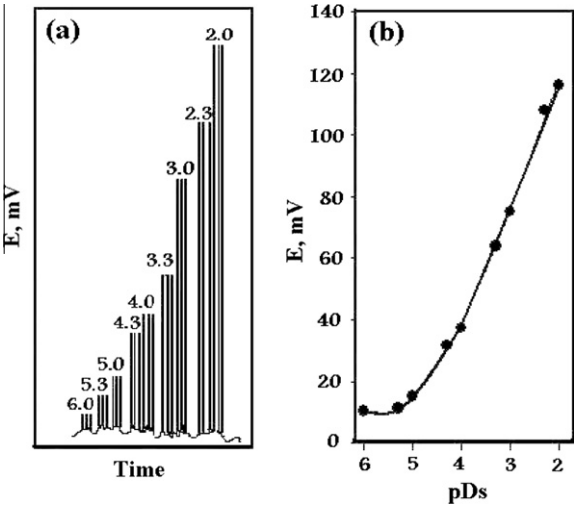

Fig. 5 The FIA recordings (a) and its corresponding calibration graphs (b) obtained for Ds-PM at optimum conditions.

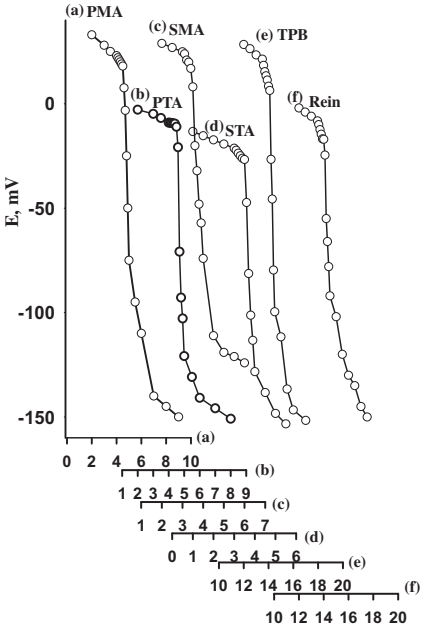

Fig. 6 Potentiometric titrations of 40.34 mg $DsBr_2$ with PMA(a), PTA (b), SMA (c), STA (d), NaTPB (e), Amm Rein (f) and picric acid (g) as titrant using Ds-PM electrode.

Table 5 Determination of distigmine bromide in pure solutions, Ubretid tablet and human urine applying the standard addition method and potentiometric titration using Ds-PM electrode.

Standard addition			Potentiometric titration[a]				
Pure solution			Pure solution[a]			Ubretid tablet (5 mg/tablet)[a]	
Taken (mg)	Recovery (%)	RSD (%)	Taken (mg)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Ds-PM electrode			*(a) PMA as titrant*				
2.88	99.8	0.15	5.76	99.5	0.45	99	0.55
5.76	99.6	0.26	17.29	100.2	0.83	100.8	0.36
17.29	99.5	0.29	28.82	100.3	0.92	100.5	0.94
28.82	99.3	0.41	40.34	100.7	1.13	100.6	0.89
Ds-PT			*(b) PTA as titrant*				
2.88	99.2	0.54	17.29	99.7	0.35	99.5	0.43
5.76	99.4	0.62	28.82	99.5	0.76	98.9	0.81
17.29	98.9	0.88	40.34	100.3	1.31	100.5	1.55
28.82	101	0.94	57.63	100.8	0.82	101	0.93
Ds-SM			*(c) SMA as titrant*				
2.88	99.7	0.22	2.88	99.8	0.52	99.3	0.36
5.76	99.5	0.27	5.76	99.5	0.74	99.5	0.45
17.29	99.4	0.5	17.29	99.6	0.98	99.3	0.36
28.82	99.1	0.65	28.82	99.3	1.05	99.1	0.88
Ds-ST			*(d) STA as titrant*				
2.88	99.5	0.29	5.76	99	0.23	99.1	0.8
5.76	98.8	0.47	17.29	99.3	0.94	99.O	0.77
17.29	101.3	0.65	28.82	99.7	0.65	99.5	0.65
28.82	98.5	0.84	40.34	100.2	0.44	100.7	0.86
Ds-PM			*(e) NaTPB as titrant*				
2.88	99.9	0.19	17.29	99.5	0.68	99.1	0.91
5.76	99.7	0.56	28.82	100.5	1.03	100.7	0.88
17.29	99.5	0.78	40.34	100.9	0.54	101.0	0.69
28.82	101.7	0.66	57.63	99	0.84	98.8	0.77
Spiked urine (Ds-PM)			*(f) Amm Rein as titrant*				
2.88	99.1	0.21	17.29	98.5	1.28	98.5	0.69
5.76	98.5	0.42	28.82	99.2	1.4	99	0.84
17.29	102	1.57	40.34	99	0.89	100.8	0.54

[a] Potentiometric titration.

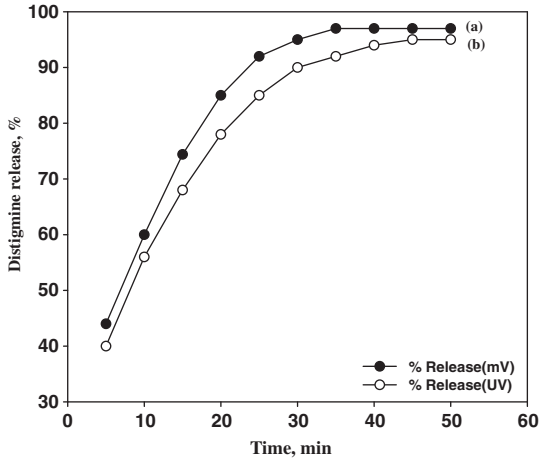

Fig. 7 Dissolution profiles of Ubretid tablet (5 mg/tablet) using potentiometric; 3.0% Ds-PM and spectrophotometric measurements.

from 98.5% to 102.0% using Ds-PM electrode, with low coefficient of variation values (0.21–1.57%). In FIA conditions, the peak heights comparison is the best method used for the distigmine determination in its pure state or pharmaceutical preparation, where the peaks obtained from a series of different concentrations of the distigmine is compared with those obtained by injecting a standard series of the distigmine measured under the same conditions of flow rate, sample volume, pH and temperature. The percentage recovery obtained ranged from 97.0% to 97.5% with the coefficient of variation values of 0.26–0.75%.

Potentiometric monitoring of distigmine tablet dissolution

The dissolution test was operated at 50 rpm in 500 ml 1.0×10^{-2} M hydrochloric acid (simulated duodenum fluid), using a distigmine ion-selective electrode. The simulated duodenum fluid was kept at 37.0 ± 0.5 °C. There are no degradation products in the in vitro test. The compression recipients do not interfere. Taking into account the S-shape of the dissolution curve obtained (Fig. 7), it is revealed that the dissolution process involves one main step, uncoated tablet dissolute. The method proved that the release of the active principle of the tablets in simulated duodenum fluid follows the Wagner model [48].

Table 6 Statistical treatment of data obtained for the determination of distigmine using Ds-electrodes in comparison with the official method.

Sample	Official method	Electrodes			FIA
		Ds-PM	Ds-SM	Ds-TPB	Ds-PM
Pure solutions					
$X \pm$ S.E.	99.8 ± 0.2	99.3 ± 0.5	99.1 ± 0.8	99.5 ± 0.2	100.3 ± 0.1
F value		1.38	2.22	3.15	3.36
t value		2.13	1.77	2.63	3.63
Ubretid tablets (5 mg)					
$X \pm$ S.E	100.3 ± 0.2	99.0 ± 0.06	98.8 ± 0.3	100.5 ± 0.1	101.7 ± 0.2
F value		2.21	1.66	3.20	3.66
t value		2.05	2.00	1.61	3.48
		Ds-PT	Ds-ST	Ds-Rein	
Pure solutions					
$X \pm$ S.E.		98.7 ± 1.0	98.5 ± 0.4	99.0 ± 0.08	
F value		4.12	2.40	2.72	
t value		3.20	1.89	2.12	
Ubretid tablets (5 mg)					
$X \pm$ S.E.		101.0 ± 0.05	100.7 ± 0.05	98.9 ± 0.06	
F value		4.55	2.10	2.58	
t value		3.41	1.25	2.30	

$X \pm$ S.E.: Recovery ± standard error.
F-tabulated is 6.39 at 95.0% confidence limit.
t-tabulated is 3.143 at 99.0% confidence limit and 6 degrees of freedom.

The potential values were continuously recorded at 1-min time intervals and compared with a calibration graph. For the UV spectrophotometric assay, fixed volumes of the dissolution medium were withdrawn, diluted with 0.01 mol L^{-1} HCl, measured at 270 ± 2 nm, and compared with a calibration graph. Fig. 7 shows the dissolution profiles of distigmine tablet using both measurement techniques. The results obtained by spectrophotometric and potentiometry are almost identical. The use of the potentiometric method sensor, however, has the advantage of in situ monitoring.

Conclusion

The proposed sensor is a novel method for the determination of distigmine bromide based on the ion-associates of Ds-ST, Ds-SM, Ds-PT, Ds-PM, Ds-TPB and Ds-Rein as modifiers for the electrodes. The electrodes are very easy to prepare, and have high sensitivity, wide dynamic range, long lifetime and very wide pH range. High selectivity and rapid response make these electrodes suitable for measuring the concentration of distigmine in a wide variety of samples (e.g. a biological sample) without the need for pretreatment steps and without significant interactions from other anionic species present in the sample. The application of the proposed method to the determination of distigmine bromide in its pure solutions and pharmaceutical preparation is characterized by a high degree of precision and accuracy when compared with the official method. The *F*- [49] and *t*-tests [50] were applied to compare the precision (coefficient of variation) and the mean values, and obtained values were much smaller than the tabulated ones, as shown in Table 6.

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