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Journal of Pharmaceutical and Biomedical Analysis 39 (2005) 117–124
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

PVC membrane ion-selective electrodes for the determination of Hyoscyamine in pure solution and in pharmaceutical preparations under batch and flow modes

Sayed S. Badawy *, Yousry M. Issa, Ali A. Mutair

Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt

Received 23 February 2005; received in revised form 29 March 2005; accepted 29 March 2005
Available online 2 June 2005

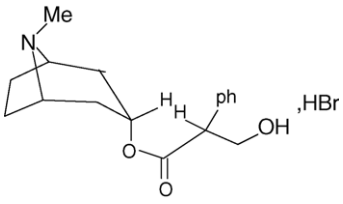
Abstract

New PVC membrane electrodes selective for the determination of hyoscyamine ion (Hy^+) based on hyoscyamine tetraphenylborate (Hy-TPB) or hyoscyamine phosphotungstate (Hy-PT) ion-exchangers as electroactive materials are described. The electrodes show a linear response for Hy^+ over the concentration range of 1.00×10^{-5} to 1.26×10^{-2} mol L^{-1} and 1.00×10^{-4} to 1.00×10^{-2} mol L^{-1} in case of Hy-TPB electrode applying batch and flow injection analysis (FIA), respectively, and 1.00×10^{-5} to 4.52×10^{-3} mol L^{-1} and 6.31×10^{-5} to 1.00×10^{-2} mol L^{-1} in case of Hy-PT electrode for batch and FIA, respectively. The lower detection limits are 3.90×10^{-6} and 4.51×10^{-6} at 25 °C for Hy-TPB and Hy-PT electrodes, respectively. The electrodes posses near Nernstian slopes of 56.5 and 57.8 mV/decade for Hy-TPB and Hy-PT electrodes, respectively, and a fast potential response of ≤20 s which is almost constant over a pH range of 3–10. Selectivity coefficient data for some common inorganic cations, sugars, amino acids and the components, other than hyoscyamine, of the mixed drugs investigated show negligible interference. The electrodes have been applied to the potentiometric determination of hyoscyamine in pure solution and in pharmaceutical preparations under batch and FIA conditions and as end point indicator electrode for the determination of hyoscyanine using potentiometric titration. For the concentrations (1.08×10^{-5} mol L^{-1} to 3.16×10^{-3} mol L^{-1}) an average recovery of 99.95% with relative standard deviation of 0.63% has been achieved. The effect of temperature on the electrodes was also studied.

Keywords: Hyoscyamine; Ion-selective electrodes; Flow injection analysis; Potentiometry

1. Introduction

Hyoscyamine (Hy) is used mainly in the relief of conditions associated with visceral spasm. It is also given for rhinitis and was formerly used in the treatment of parkinsonism [1].



Several methods have been reported for the quantitative determination of Hy in pure solutions and/or in pharmaceutical preparations. Among these are high performance liquid chromatography (HPLC) [2], reversed phase high performance liquid chromatography [3], thin layer chromatography (TLC) [4], liquid chromatography (LC) [5], gas chromatography–mass spectroscopy (GC–MS) [6], liquid chromatography–mass spectroscopy (LC–MS) [7], micellar electrokinetic chromatography and mass spectroscopy (MS) [8], capillary electrophoresis [9–11] and spectrophotometry [12]. Most of these methods involve several time-consuming manipulation steps and require sophisticated instruments.

Potentiometric methods using ion-selective electrodes have found wide applications [13–15], due to their high precision, rapidity, low cost of analysis, no sample pretreatment

* Corresponding author. Tel.: +20 101804728; fax: +20 2 5728843.
E-mail address: ssbadawy@chem-sci.cu.edu.eg (S.S. Badawy).

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doi:10.1016/j.jpba.2005.03.025

needed before the analysis itself, and selectivity [16]. However, no work has been done on the development of ISEs for the determination of Hy which have considerable applications in the laboratories of drug control and in many other biological and chemical research areas, specially when they are applied in FIA mode allowing the in vivo measurements. Mostafa [17] reported a poly vinyl chloride (PVC) membrane sensor for the potentiometric determination of scopolamine HBr, which is of approximate similar structure as hyoscyamine but not identical and each of them has its own medicinal action and pharmaceutical preparations. Recently, scopolamine is prepared from hyoscyamine by biosynthesis [18,19]. The reported electrode was based on scopolamine–phosphotungstate ion pair as an electroactive material, and applied only for the potentiometric determination of scopolamine in pharmaceutical preparations with performance characteristics similar to or, in some times, less than those of the present work.

In the present work, two ISEs based on incorporation of hyoscyamine-tetraphenylborate (Hy-TPB) or hyoscyamine-phosphtungstate (Hy-PT) ion-exchangers in PVC membrane plasticized with dioctylphthalate (DOP) or dibutylphthalate (DBP). The proposed electrodes have been successfully used for the determination of Hy both in batch and in flow injection analysis (FIA) situations.

2. Experimental

2.1. Reagents

All reagents were analytical reagent grade. Double-distilled water was used for the preparation of all solutions and as flow stream in FIA measurements. Hyoscyamine hydrobromide (Hy·Br), sodium tetraphenylborate (NaTPB), phosphotungstic acid (PTA), DOP and DBP were Fluka products, while PVC of high molecular mass and tetrahydrofuran (THF) were Aldrich products. Sanzyme “2000”, atropine sulphate (atropine sulph.) and phenobarbital were kindly provided, as a gift, from National Organization for Drug Control and Research, Giza, Egypt. Pharmaceutical preparations were purchased from local drug stores, information concerning their composition is given in Table 1.

In FIA measurements, the carrier and reagent solutions were degassed by means of vacuum suction. Sample solutions were freshly prepared prior to measurements.

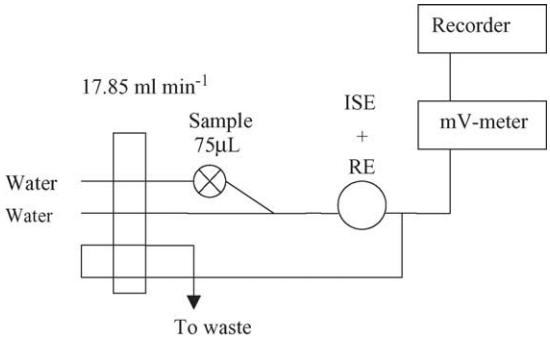

Fig. 1. Schematic diagram of the flow injection system used in the measurements.

2.2. Instrument

Potentiometric and pH measurements in the batch mode were carried out by using Metrohm titroprocessor model 682. A techne circulator thermostat model C-100, was used to control the temperature of the test solutions. A double junction Ag/AgCl/ 3 mol L^{-1} KCl reference electrode model Metrohm 60733.100 was used as the external reference electrode and a Ag/AgCl wire as the internal reference.

The flow injection setup is composed of a four-channel peristaltic pump (Ismatec, ISM 827, Zurich, Switzerland), injection valve model 5020 with exchangeable sample loop from Rheodyne (Cotati, CA, USA). The electrodes were connected to a WTW pMX 2000 microprocessor pH/ion meter and interfaced to a model BD111 strip chart recorder (Kipp and Zonn Deflt, The Netherlands).

A wall-jet cell, providing a low dead volume, fast response, good wash characteristics, ease of construction and compatibility with the electrodes of the different shapes and sizes, was used in the flow measurements, where a Perspex cup with axially positioned inlet polypropylene tubing was mounted at the sensing surface of the electrode body. The ion-selective electrode with flow cup, reference electrode and the outlet tubes were placed in a beaker, where the level of the solution was kept 1 cm above the electrode surface. Fig. 1 represents a scheme of the flow injection system used in the measurements.

2.3. ISE preparation and potentiometric measurements

The Hy-TPB and Hy-PT ion-exchangers and Hy-TPB and Hy-PT sensitive electrodes were prepared as described

Table 1
Pharmaceutical preparations of the investigated drug

Trade name	Composition	Company
Donalase-S (tablets)	Sanzyme “2000” (85 mg/tab.) Hyoscyamine sulphate (0.10 mg/tab.) Hyoscine HBr (0.007 mg/tab.) Atropine sulphate (0.020 mg/tab.) Phenobarbital (16.2 mg/tab.)	Misr Co. for pharm. Ind., S.A.A. Mataria Cairo, A.R.E.
Neo-Allospasmin (drops)	Hyoscyamine sulphate (0.125 mg/ml)	The Arab Pharmaceutical Manufacturing Co. Ltd., Sult, Jordan

previously [20]. The composition of the ion-exchangers was found to be 1:1 in case of Hy-TPB and 3:1 in case of Hy-PT, as confirmed by elemental analysis data done at the Microanalytical Center, Cairo University, Egypt. The values found are 80.80, 7.30, and 2.14 and the calculated are 80.71, 7.28, and 2.29 for C, H, and N%, respectively, in case of Hy-TPB, while in case of Hy-PT the values found are 17.01, 2.00, and 1.12 and the calculated are 16.34, 1.94, and 1.12 for C, H, and N%, respectively. The membrane contained 17.50 mg Hy-TPB ion-pair, 166.25 mg PVC and 162.93 ml DOP in case of Hy-TPB electrode and 35 mg Hy-PT ion-associate, 157.50 PVC and 160.65 ml DBP in case of Hy-PT electrode. The membrane components (totaling 350 mg) were dissolved in THF (10 ml) and poured into a 7.5 cm Petridish. Overnight evaporation of the solvent yielded a membrane of ≈0.1 mm thickness, visually determined by an optical microscope. For each electrode, 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. The electrodes were then filled with 0.1 mol L^{-1} NaCl + 10^{-2} mol L^{-1} Hy·Br solution and the Ag/AgCl wire was immersed in this solution. The resulting electrodes were preconditioned by soaking for 2 h in 10^{-3} mol L^{-1} Hy·Br solution. The electrochemical system is composed as follows:

Ag/AgCl/inner solution/membrane/test solution//KCl salt bridge//Ag/AgCl/3 mol L^{-1} KCl.

2.4. Calibration of the electrodes

For batch measurements, suitable increments of standard (Hy·Br) solution were added to 50 ml double-distilled water to cover the concentration range (1.00×10^{-5} to 4.52×10^{-3} M). In this solution, the sensor and the reference electrodes were immersed and after each addition the emf was recorded at 25 ± 1 °C. The cell potentials, E_{cell}, were recorded and plotted versus log [Hy·Br].

For FIA measurements, a series of freshly prepared solutions of the Hy·Br covering the range (1.0×10^{-5} to 1.0×10^{-2} M) was injected to the flow stream and the corresponding peak heights were recorded and used to draw the calibration graphs.

2.5. Potentiometric determination of hyoscyamine

In batch measurements, the standard addition method was applied in which small increments of Hy·Br solution (0.10 M) were added to 50 ml aliquot samples of various concentrations ($1.62 \times 10^{-4} - 2.70 \times 10^{-3}$ M). The change in the potential reading was recorded for each increment and used to calculate the concentration of Hy·Br in the sample solution using the following equation:

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

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

For analysis of pharmaceutical preparations, appropriate weights or volumes from ground tablets (Donalase-S) or drops (Neo-Allospasmin), respectively, containing 3.00, 5.00, 10.00, 20.00 and 30.00 mg of hyoscyamine were taken as samples and dissolved in 50 ml of double-distilled water and then the standard addition technique was applied as described above.

In FIA, three series have been prepared. The first was for a standard Hy·Br, the second for tablets and the third for drops. The peak heights for each series were measured and then used for calculating the recovery percent of hyoscyamine in tablets and drops.

In potentiometric titration of hyoscyamine, aliquot of Hy·Br containing 6.00–50.00 mg was transferred into the titration cell and the solution was diluted to 50 ml with double-distilled water. The resulting solution was titrated with 10^{-2} mol L^{-1} NaTPB or PTA solutions. The same method was applied for pharmaceutical preparations. It is important to notice that titrations were performed in non-flow mode.

3. Results and discussion

3.1. Electrodes performance

3.1.1. In batch conditions

Six membrane compositions were prepared by varying the percentage of the ion-exchangers (Hy-TPB or Hy-PT) while keeping the percentage of the PVC and the plasticizer equal (1:1). The results showed that the electrodes containing 5% (w/w) of Hy-TPB or 10% (w/w) Hy-PT gave near Nernstian slopes (56.5 and 57.8 mV per concentration decade, respectively) over a relatively wide concentration range of Hy·Br (1.00×10^{-5} to 1.26×10^{-3} M and 1.00×10^{-5} to 4.57×10^{-3} M), the detection limits were found to be 3.90×10^{-6} and 4.51×10^{-6} mol L^{-1} for Hy-TPB and Hy-PT electrodes, respectively [21]).

Additional preliminary experiments demonstrated that the sensitive membrane electrodes were stable over a one month period of continuous soaking and then a significant shift in the response toward Hy^+ was observed. It can be assumed that the concentration of the plasticizer and the ion-exchanger in the membrane is likely to be reduced during this contact period with the bathing solution and the PVC tubing. This reduction is likely due to the leaching of the ion-exchangers into the bathing solution and due to the diffusion of the plasticizer and ion-exchanger from the membrane into the PVC tubing [22].

The effect of solution temperature on the response of the membrane electrosdes were studied at different temperatures.

The electrodes gave good Nernstian response over the temperature range 25–60 °C. The standard electrode potentials, E°, were determined at different temperatures and used for calculation of the thermal coefficients of the electrodes [20], which were found amounting to 5.20×10^{-4} and 1.13×10^{-3} V/°C for Hy-TPB and Hy-PT membrane electrodes, respectively, revealing a fairly good thermal stability of the two electrodes.

3.1.2. In FIA mode

The flow injection measurements were carried out in a two-line system; the sample was injected into a distilled water stream, which then merged with another stream of distilled water. In both lines, the same tubing size was used, offering the same flow rate. Fig. 1 shows the configuration of the system used in the measurements. This configuration was used to study the effect of pH, ionic strength, interference and other experimental factors without apparent dilution as indicated by the low dispersion coefficient values amounting to 1.31 and 1.34 for Hy-TPB and Hy-PT electrodes, respectively, i.e. limited dispersion that aids optimum sensitivity and fast response of the electrodes [23].

3.1.2.1. Effect of injection volume. Samples of different volumes (4.70–340.0 μl) were injected. In general, higher the sample volume, higher the peak heights and residence time of the sample at the electrode surface thus takes a longer time to reach a steady state and higher consumption of the sample [24]. A sample loop of size 75.0 μl was used throughout this work giving maximum peak height, less consumption of the reagents, and a shorter time to reach the base line.

3.1.2.2. Effect of flow rate. The dependence of the peak heights and time to recover the base line on flow rate was investigated, where the response of the two electrodes to a solution that is 10^{-3} mol L^{-1} was tried at different flow rates (4.1, 5.3, 7.5, 9.7, 12.5, 17.8, 23.2, 25.0, 27.0 and 30.0 ml/min) with constant injection volume (75.0 μl). The residence time of the sample is inversely proportional to the flow rate of the sample at the active membrane surface. The results showed that, as the flow rate increases, the peaks become higher and narrower until a flow rate of 17.85 ml/min where the peaks obtained after that, at higher flow rates, are nearly the same. This rate was used throughout this work providing the maximum peak height, a shorter time to reach the base line and less consumption of the carrier solution.

3.2. Electrode response in FIA

In potentiometric detection, the electrode potential depends on the activity of the main ion sensed, but in flow measurement, the main unfavorable feature of this detection is the slow response of electrode potential to concentration change, which is pronounced when low concentrations are measured and depends on the state of the membrane surface at the interface with the measured solutions [25]. This slow response is a quite good reason for the super Nernstian sensitivities obtained in FIA measurements using the investigated electrodes. An increase in the slope of the calibration plots in FIA was observed compared to batch measurements, where the potential is measured in conditions very close to the equilibrium at membrane solution interface. The slopes of the calibration graphs obtained are 58.1 and 60.0 compared to 56.5 and 57.8 mV per concentration decade in batch conditions for Hy-TPB and Hy-PT electrodes, respectively. The usable concentration ranges of the electrodes in FIA measurement are (1.00×10^{-4} to 1.00×10^{-2} M), for Hy-TPB electrode and (6.31×10^{-5} to 1.00×10^{-2} M) for Hy-PT electrode. Fig. 3 (a_1 and a_2) represents the recorded peaks and Fig. 3 (b_1 and b_2) represents the calibration graphs for Hy-TPB and Hy-PT electrodes at the optimum conditions. Collective data of the response characteristics of the investigated electrodes were given in Table 2.

3.3. Effect of pH

The electrodes response for different Hy·Br concentrations was tested at different pH values, the pH being adjusted using hydrochloric acid and/or sodium hydroxide solutions. The pK_a values for HyH^+ is 10.5 as determined practically under our experimental conditions (the cited value amounts to 9.7 for hyoscyamine and 7.6 for scopolamine [26]). In Fig. 2, the potential of the proposed electrodes dipped into different Hy·Br solutions of 5×10^{-3}, 5×10^{-4} and 5×10^{-5} mol L^{-1} concentration is plotted against the pH of the solution. From this figure it is apparent that the electrode response is almost stable within ±2 mV over the pH range of 3.5–10.0. It also clear that the protonated form will exist in 50% portion up to about pH 10.5.

3.4. Response time

The time required reaching a steady potential within ±1 mV, after successive immersion of the electrodes in different concentrations of Hy·Br solutions each having a tenfold difference in concentration has been measured. The average

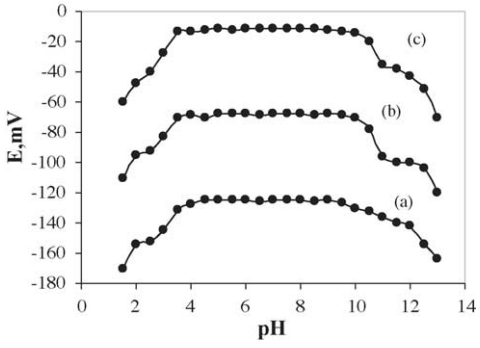

Fig. 2. Effect of pH of the test solution of concentrations 5.0×10^{-5} (a), 5.0×10^{-4} (b), and 5.0×10^{-3} mol L^{-1} (c) Hy·Br on the potential response of Hy-PT electrode.

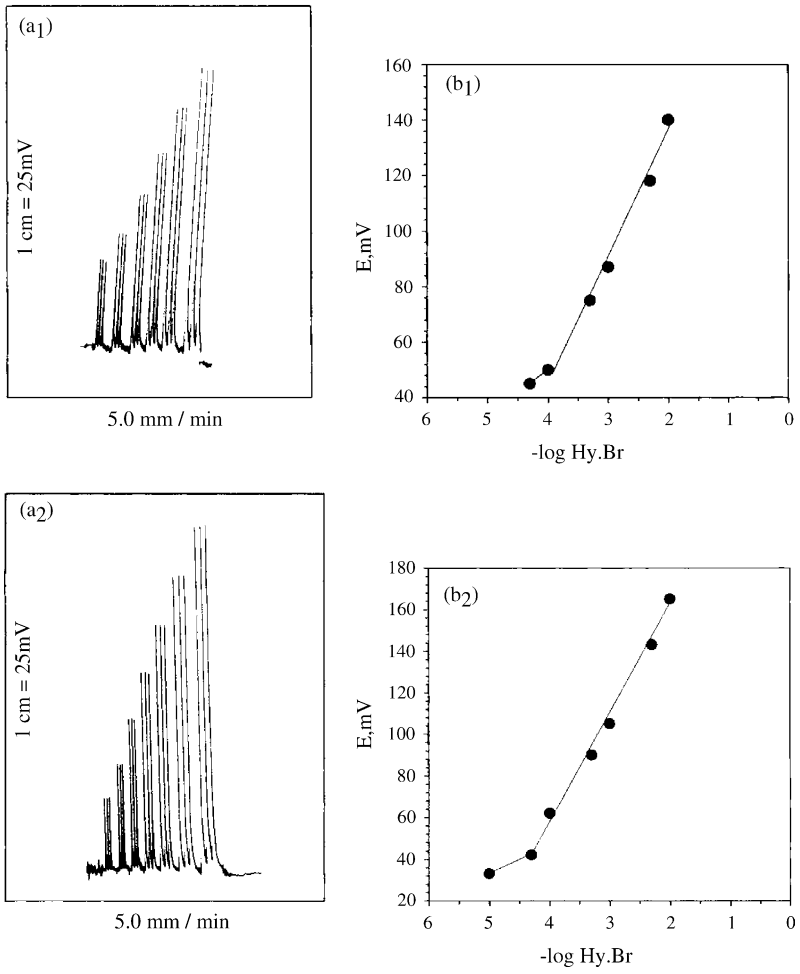

Fig. 3. Recordings (a_1 and a_2) and their corresponding calibration graphs (b_1 and b_2) obtained for Hy-TPB and Hy-PT electrodes at optimum FIA conditions.

dynamic response time was found to be short, ≤20 s. Inter-day-precision of the electrodes is about 2 mV for the same solution and the useful lifetime of Hy-TPB and Hy-PT electrodes are 25 and 34 days, respectively, during which the potential slopes are repeatable to within ±1 mV/concentration decade. Also after more than 2 months a new section from the master membrane was found to function very properly.

3.5. Selectivity of the electrodes

In batch measurements, the selectivity coefficients $K^{\text{pot}}_{D,J^{z+}}$ were evaluated by the separate solution method SSM [21] for ions with charge numbers, where the following equation was applied:

$$\log K^{\text{pot}}_{D,J^{z+}} = \frac{E_2 - E_1}{S} + \log[D] - \log[J^{z+}]^{1/z}$$

where E_1 is the electrode potential in 10^{-2} mol L^{-1} Hy·Br solution, E_2 the potential of the electrode in 10^{-2} mol L^{-1} interferent solution J^{z+} and S is the slope of the calibration graph.

The $K^{\text{pot}}_{D,J^{z+}}$ was also determined by the matched potential method MPM [27] for all types of ions and molecules. In this method, the potentiometric selectivity coefficient is

Table 2
Response characteristics of the hyosyamine electrodes

	Hy-TPB electrode		Hy-PT electrode	
	Batch	FIA	Batch	FIA
Electrode composition (w/w)	5% Hy-TPB ion-pair, 47.5%, PVC, 47.5% DOP		10% Hy-TPB ion-exchanger, 47.5%, PVC, 47.5% DBP	
Slope (mV/decade)	56.5	58.1	57.8	60.0
Lower detection limit (mol L^{-1})	3.90×10^{-6}	1.00×10^{-4}	4.51×10^{-6}	6.31×10^{-5}
Response time s	≤20	≤20	≤20	≤20
Working pH range	3.5–9.0	3.5–9.0	3.5–10.0	3.5–10.0

Table 3
Selectivity coefficients $K^{pot}_{D,J^{+z}}$ for the Hy-electrodes in batch and FIA conditions

Interferent	Hy-TPB electrode			Hy-PT electrode		
	Batch		FIA	Batch		FIA
	SSM	MPM		SSM	MPM	
Na^+	7.37×10^{-3}	4.29×10^{-4}	5.18×10^{-3}	4.38×10^{-3}	3.07×10^{-4}	1.70×10^{-3}
K^+	5.26×10^{-3}	1.35×10^{-3}	3.16×10^{-3}	1.37×10^{-2}	5.43×10^{-4}	4.12×10^{-3}
NH_4^+	4.44×10^{-3}	4.86×10^{-4}	1.74×10^{-3}	9.55×10^{-3}	4.39×10^{-4}	3.11×10^{-3}
Li^+	6.23×10^{-3}	1.19×10^{-3}	3.55×10^{-3}	1.74×10^{-3}	3.26×10^{-4}	7.02×10^{-4}
Mg^{2+}	8.16×10^{-4}	2.06×10^{-3}	2.28×10^{-5}	8.71×10^{-5}	2.04×10^{-4}	3.71×10^{-5}
Ca^{2+}	4.29×10^{-3}	2.14×10^{-3}	4.97×10^{-4}	2.75×10^{-4}	2.63×10^{-4}	1.43×10^{-4}
Sr^{2+}	2.40×10^{-4}	3.76×10^{-4}	9.21×10^{-5}	8.38×10^{-5}	1.83×10^{-4}	2.88×10^{-5}
Ba^{2+}	1.82×10^{-3}	1.10×10^{-3}	1.71×10^{-4}	1.38×10^{-4}	2.41×10^{-4}	1.07×10^{-4}
Mn^{2+}	8.73×10^{-4}	4.37×10^{-4}	3.58×10^{-5}	1.66×10^{-4}	2.32×10^{-4}	1.53×10^{-4}
Co^{2+}	7.89×10^{-4}	3.52×10^{-4}	3.03×10^{-5}	1.00×10^{-4}	1.76×10^{-4}	7.59×10^{-5}
Ni^{2+}	9.77×10^{-4}	3.08×10^{-4}	2.47×10^{-5}	4.43×10^{-4}	2.43×10^{-4}	1.19×10^{-5}
Cu^{2+}	7.37×10^{-4}	2.41×10^{-4}	5.62×10^{-5}	4.12×10^{-5}	2.89×10^{-4}	1.38×10^{-5}
Zn^{2+}	5.62×10^{-4}	4.42×10^{-4}	1.94×10^{-5}	4.43×10^{-5}	2.07×10^{-4}	1.58×10^{-5}
Pb^{2+}	3.16×10^{-3}	5.59×10^{-4}	2.57×10^{-4}	6.12×10^{-3}	3.75×10^{-4}	3.46×10^{-4}
Cd^{2+}	2.86×10^{-3}	5.22×10^{-4}	2.57×10^{-4}	7.53×10^{-5}	1.64×10^{-4}	3.80×10^{-5}
Hg^{2+}	1.23×10^{-3}	3.21×10^{-4}	9.21×10^{-4}	2.36×10^{-5}	1.15×10^{-4}	1.53×10^{-5}
Al^{3+}	4.06×10^{-4}	4.47×10^{-4}	1.53×10^{-4}	5.58×10^{-4}	4.17×10^{-4}	5.54×10^{-5}
Cr^{3+}	4.48×10^{-4}	7.05×10^{-4}	1.15×10^{-4}	3.21×10^{-4}	8.47×10^{-4}	4.99×10^{-5}
Fe^{3+}	5.75×10^{-4}	7.31×10^{-4}	1.35×10^{-4}	1.48×10^{-4}	6.3410^{-4}	1.21×10^{-5}
Glucose	–	$<10^{-5}$	–	–	$<10^{-5}$	–
Fructose	–	$<10^{-5}$	–	–	$<10^{-5}$	–
Maltose	–	2.20×10^{-4}	–	–	$<10^{-5}$	–
Lactose	–	$<10^{-5}$	–	–	$<10^{-5}$	–
Alanine	–	$<10^{-5}$	–	–	$<10^{-5}$	–
Arginine	–	$<10^{-5}$	–	–	$<10^{-5}$	–
Glycine	–	$<10^{-5}$	–	–	1.40×10^{-4}	–
Threonine	–	$<10^{-5}$	–	–	1.67×10^{-4}	–
Phenobarbital	–	$<10^{-5}$	–	–	$<10^{-5}$	–
Sanzyme '2000'	–	$<10^{-5}$	–	–	$<10^{-5}$	–
Atropine sulphate	–	$<10^{-5}$	–	–	$<10^{-5}$	–
Scopolamine	–	$<10^{-5}$	–	–	$<10^{-5}$	–

SSM: separate solution method, MPM: matched potential method.

defined as the activity ratio of primary and interfering ions that give the same potential change under identical conditions. At first, a known concentration (C'_D) of the primary ion solution is added into a reference solution that contains a fixed concentration (C_D) of primary ions, and the corresponding potential (ΔE) is recorded. Next, solution of an interfering ion is added to the reference solution until the same potential change (ΔE) is recorded. The change in potential produced at the constant background of the primary ion must be the same in both

$$K^{pot}_{D,J^{z+}} = \frac{C'_D}{C_J}$$

where C_J is the concentration of the interfering ion.

In FIA measurements, the sample remains in contact with the electrode for a short period of time, consequently, the apparent selectivity is expected to be different from that found in batch conditions. In this case, the values of selectivity coefficients $K^{pot}_{D,J^{z+}}$ were calculated based on potential values corresponding to the peak heights for the same concentrations of the drug and the interferent.

The determined selectivity coefficients (Table 3) reflect a very high selectivity of the investigated electrodes toward hyoscyamine cation, under both batch and FIA conditions, with respect to sanzyme "2000", atropine sulphate and phenobarbital that are mixed with hyoscyanine in pharmaceutical preparations mentioned in Section 2, in addition to many common inorganic cations, sugars and amino acids which are frequently present in biological fluids and pharmaceutical preparations. The inorganic cations did not interfere due to the differences in their mobilities and permeabilities as compared to Hy^+ ions.

4. Analytical applications

Hyoscyamine was determined, in pure solutions and in pharmaceutical preparations, potentiometrically using the investigated electrodes under batch conditions by both standard addition method and potentiometric titration.

The mean recovery and the relative standard deviation values, for pure solution and pharmaceutical preparations,

Table 4
Determination of hyoscyamine in pure form and in pharmaceutical preparations by applying standard addition and potentiometric titration methods under batch and FIA conditions

	Taken[a] (mg)	Hy-TPB electrode		Hy-PT electrode	
		Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
Standard addition method					
Pure solution	3.00–50.00	99.53–101.27	0.21–0.96	98.54–103.15	0.37–0.85
Donalase-S (tablets)	3.00–30.00	99.14–101.13	0.25–1.15	99.44–103.11	0.43–1.64
Neo-Allospasmin (drops)	3.00–18.75	96.99–99.76	0.55–1.23	97.93–101.17	0.83–1.15
Potentiometric titration[b]					
Pure solution	6.00–50.00	98.75–101.31	0.24–0.53	99.36–103.10	0.39–0.68
Donalase-S (tablets)	6.00–30.00	97.05–100.23	0.27–0.65	97.74–101.35	0.37–0.74
Neo-Allospasmin (drops)	6.00–18.75	96.00–99.98	0.39–1.25	100.68–103.27	0.40–0.81
FIA method					
Donalase-S (tablets)	0.19–58.51	95.44–100.13	0.21–0.57	98.29–101.61	0.22–0.64
Neo-Allospasmin (drops)	0.19–9.26	95.20–100.04	0.32–0.60	96.00–99.35	0.43–0.84

[a] Taken (mg/50 ml) are these values that lie within the linear part of the calibration graphs.
[b] Experiments were performed in non-flow mode.

Table 5
Statistical treatment of the data obtained for hyoscyamine using Hy-electrodes as compared with the results obtained by applying the official method [28]

Electrode	Method	*S*	*I*	*R*	*F*-value (6.39)[a]	*t*-value (5.04)[b]
Hy-TPB	Potentiometric titration[c]	0.999	−0.031	0.999	2.95	1.86
	Standard additions	0.998	0.220	0.998	3.09	2.95
	FIA	0.988	−0.045	0.994	1.93	0.84
Hy − PT	Standard additions	0.997	−0.016	0.999	2.48	2.57
	Potentiometric titration[c]	0.994	0.150	0.998	2.81	1.96
	FIA	0.995	−0.064	0.999	1.75	0.91

S: slope of the regression line of mg taken versus mg found. *I*: intercept of the regression line. *R*: correlation coefficient of the regression line.
[a] *F*-tabulated at 95% confidence limit.
[b] *t*-tabulated at 99.9% confidence limit.
[c] Experiments were performed in non-flow mode.

were calculated and summarized in Table 4. In pharmaceutical analysis, it is important to test the selectivity toward the components of the mixed drugs preparations, other than analyte (Hy·Br), and the excipients and fillers added to the pharmaceutical preparations. It is clear from the results obtained for pharmaceutical preparations (Table 4) that these components and excipients do not interfere as indicated by high recovery and low standard deviation values.

Under FIA conditions, a series of solutions of different concentrations was prepared from tablets and drops and the peak heights were measured at the optimum conditions then compared to those obtained from injecting a standard solution of the same concentration prepared from pure Hy·Br. The mean recovery values for the amounts taken (0.19–58.51 mg) ranged from 95.20 to 100.68% (Table 4). The recovery values obtained using FIA conditions were relatively lower than those obtained under batch conditions.

The results of the proposed methods, batch and FIA, indicate the high accuracy and precision of the present work (Table 4). These results were compared with those obtained from the official method (based on non aqueous titration of Hy·Br with 0.1 N of perchloric acid in the presence of mercuric acetate and using crystal violet as indicator) [28] by applying *F*- and *t*-tests [29]. The calculated *F*-values were found to be in the range 1.75–3.09, which are lower than the tabulated value (6.39 at 95% confidence limit and 4 d.f.) while, the *t*-values were found in the range 0.84–2.95, which are lower than the tabulated value at 99.9% confidence limit and 8 d.f. (5.04). This means that the present methods are of comparable precision to that of the official method and there is no significant difference between the mean values obtained by both methods. In order to establish whether the proposed methods exhibit any fixed or proportional bias, a simple linear regression of the taken (milligrams) against found was calculated, and the results of statistical treatments of the data are shown in Table 5.

5. Conclusion

The proposed electrodes based on Hy-TPB or Hy-PT with PVC conventional type, offer a valuable technique for the determination of hyoscyamine in pure solutions and in pharmaceutical preparations using batch and FIA conditions. The inherent advantages of the proposed electrodes are its rapid response, simple operation, precise results, low cost and direct application to the determination of hyoscyamine in complex matrix without prior separation. The proposed electrodes

characterized by high selectivity toward hyoscyamine than other reported methods, they can be introduced commercially, facilitating Hy·Br determination in non-specialized laboratories and the present work shows the applicability of the developed electrodes in flow injection systems for the determination of Hy·Br by direct potentiometry in a short time.

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