

Sensors Membrane Electrodes for Sensitive Determination of *Hyoscine Butylbromide* in Pharmaceutical Formulation and in Human Plasma

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Abstract: The proposed sensors of the construction of *Hyoscine Butylbromide* (HyB) ion-selective electrodes based on the complex ion associate of hyoscine with tetraphenyl borate ionophor in the polyvinyl chloride (PVC) matrix with dioctyl phtalate and dibutyl sebatate as plasticizers. The electrochemical performance characteristics of the two HyB-selective electrodes were evaluated according to IUPAC standards. Sensors show stable potential response with near Nernstian slope of 52.42, 54.57 mV per decade for sensors denoted as № 1 and 2, respectively. Selectivity coefficients data for different organic and inorganic ions are also presented. The two sensors have fast response time (20-30s) and applicable over a wide range of the operational pH (6-10). The results obtained from the determination of HyB with the two proposed electrodes have shown the average recoveries of 98.6%, 99.6% for analysis of tablets and 100.17%, 99.01 for (spiked) human plasma. The data agree well with those obtained by the standard reference methods.

Keywords: Direct potentiometry; PVC sensors; *Hyoscine Butylbromide* (HyB); Ion-associate with tetraphenyl borate, Determination, Human plasma.

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Introduction

Hyoscine Butylbromide (HyB), having the formula (1S,3S,5R,6R,7S,8r)-6,7-epoxy-8-butyl-3-[(S)-tropoyloxy]tropanium bromide [1]), is being used as antispasmodic in treating peptic ulcer, gastritis, and other disorders of gastrointestinal tract which are characterized by spasm. It has also found the applicability in the relief of spasmodic of dysmenorrhoea [2].

Several methods have been reported for its determination in pharmaceutical formulations and in biological fluids including spectroscopic methods [3-12], electrochemical methods [13-18], and chromatographic methods [19-24].

The present work originates from the fact that HyB behaves as a cation of the quaternary ammonium type. This fact suggests the use of anionic ion-exchangers, forming water insoluble ion-association complexes. Tetraphenyl borate as an anionic exchanger, was used for preparation of water insoluble ion-associate with HyB. The high lipophilicity and remarkable stability of this complex suggested its selective use as electroactive materials in PVC matrix membrane sensors for the determination of HyB alone in plasma and in pharmaceutical dosage forms in combination with *Paracetamol* without interference.

Experimental

Chemicals and Reagents

All the chemicals used were of analytical grade: (i) tetrahydrofuran (THF, 99%; Lab Scan); (ii) high-molecular weight polyvinyl chloride (PVC, powdered form; M.w.: 10000 g·mol⁻¹); (iii) nitrophenyloctyl ether (o-NPOE) were obtained from Aldrich; (iv) dibutyl sebacate and tetraphenyl borate (both as fine chemicals from Sigma); (v) phosphate buffer (pH 8; 0.191 g KH₂PO₄ + 2.300 g Na₂HPO₄ in 1000 mL water; prepared according to [25]). All solutions were made from doubly distilled water.

Apparatus and Other Accessories

Potentiometric measurements were made with a pH /mV meter (Hanna, model 211). A single junction reference calomel electrode (SCE, model HI 5412) was used in conjunction with the drug sensor and a pH combined glass electrode (WPA, model CD 740). Another equipment was a sonication bath (Bandelin, model RK 510-S), and a magnetic stirrer.

Samples

Pure Samples ... Hyoscine Butylbromide (HyB) and Paracetamol (PA) were kindly supplied by Chemical Industries Development, Co., Cairo, Egypt. Their purity was found to be 100.02 % and 99.90%, respectively (in accordance with the declaration by the manufacturer).

Solutions

Stock Standard Solutions ... 0.01 M HyB stock solution in either pure water or phosphate buffer (PhB, pH 8) were prepared by transferring 0.4404 g HyB into two separate 100-mL measuring flasks. Then, 50 mL either water or PhB were added, shaken for few minutes, and made up to volume with the same solvent.

Working Standard Solutions ... HyB working solutions (in concentration range of 1×10^{-7} – $0.001 \text{ mol} \cdot \text{L}^{-1}$) were prepared by appropriate diluting from the respective stock solution using either water or PhB.

Laboratory-Prepared Mixtures ... 2.5 mL HyB from its 0.01 M stock solution were transferred accurately to a series of 25-mL measuring flasks. Aliquots from 0.01 M PA solution were added to prepare mixtures containing 1:1, 1:0.5 and 1:2 of HyB and PA, respectively.

Pharmaceutical Dosage Form Sample Solutions ... (1) *Buscopan-plus*[®] tablets: Ten tablets were accurately weighed and finely pulverized. An amount of the powder equivalent to 22.02 mg HyB was accurately transferred to a 50-mL volumetric flask and the volume was completed to the mark with phosphate buffer pH 8 to prepare 10^{-3} M solution of HyB. (2) *Buscopan-plus*[®] suppositories: Two suppositories were weighed and melted in 100-mL beaker. They were left to solidify then an amount equivalent to 22.02 mg of HyB from the melted suppositories was accurately transferred into 100-mL beaker and 25.0 mL PhB was added and warmed to dissolve with mixing, allowed to cool in refrigerator then filtered through filter paper into 50-mL volumetric flask. The volume was then completed to the mark with PhB to yield the resultant solution of 0.001 M HyB.

Human Plasma Samples ... 4.5 mL of plasma were placed into 2 stopped shaking tube, then 0.5 mL 0.01 and 0.001 M HyB were added separately and shaken in order to prepare $10 \times$ diluted solutions of HyB.

Pre-analysis Procedures

Preparation of Hyoscine Membrane Sensors ... (A) Preparation of HyB-tetraphenyl borate ion-exchanger: 10.0 mL 0.01 M HyB aqueous solution were mixed with 10.0 mL of a saturated solution of tetraphenyl borate. The resulting precipitate was filtered, washed with cold water, dried at room temperature, and grinded to a fine powder. Elemental CHN-analysis (for carbon, hydrogen and nitrogen) was carried to study the formation of the complex ratio.

(B) HyB-PVC membrane sensors: In two glass Petri dish (5 cm diameter), 10.0 mg of the previously prepared ion association complex was mixed thoroughly with 0.35 mL of either dibutylsebacate (sensor № 1) or nitrophenyl octyl ether (sensor № 2) then add 0.19 g of poly vinyl chloride (PVC). These mixtures were dissolved in 5.0 mL tetrahydrofuran (THF), cover the dishes with a filter paper and leave to stand overnight to allow slow evaporation of the solvent at room temperature forming master membrane with 0.1 mm thickness.

(C) Sensors assembly: Sensors were assembled using a disk of an appropriate diameter (about 8.0 mm) were cut from the previously prepared master membranes and cemented to the flat end of PVC tubing with THF. A mixed solution consisting of equal volumes of 0.01 M HyB and 0.01 M NaCl was used as an internal reference solution. Ag/AgCl coated wire (3.0 mm in diameter) was employed as the internal reference electrode. The sensors were conditioned by soaking for 24 hour in a solution of 0.01 M of drug and stored in the same solution unless in use. The overall assembly of the ion-selective electrode (ISE) is illustrated in Fig. 1.

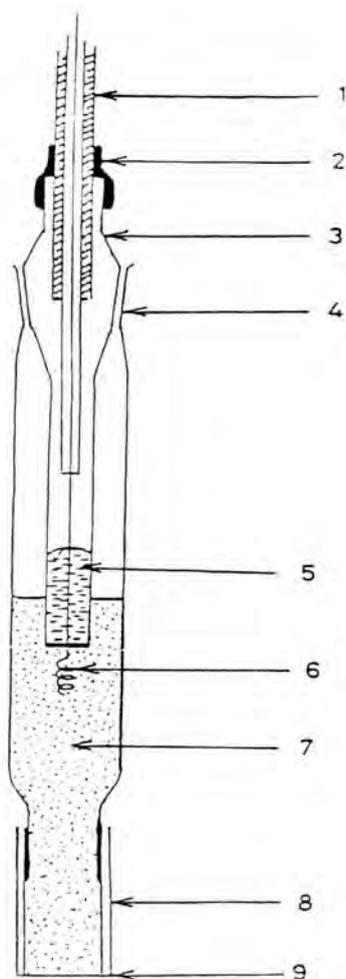


Figure 1.

PVC-matrix membrane ion-selective electrode:

- [1] shielded cable
- [2] rubber sheath;
- [3] quickfit cone;
- [4] quickfit socket;
- [5] mercury filling;
- [6] Ag/AgCl electrode;
- [7] internal solution;
- [8] PVC tubing
- [9] sensor membrane

Analytical Procedures

Application to Laboratory Prepared Mixtures. The membrane sensor was immersed in conjunction with the single junction calomel reference electrode in the different laboratory prepared mixtures. The membrane sensor was washed with water between measurements. The e.m.f. produced for each mixture was measured by the two proposed electrodes then the concentration of HyB was determined from the corresponding regression equation.

Application to Pharmaceutical Dosage Form. The e.m.f. produced by immersing the prepared electrodes in conjunction with single junction calomel reference electrode in the prepared solutions of buscopan plus tablets and suppositories were determined then the concentration of HyB was calculated from the regression equation of the corresponding electrode.

Application to Plasma Samples. The membrane sensor was immersed in conjunction with the single junction reference calomel electrode in spiked plasma solutions. The membrane sensor was washed with water between measurements. The e.m.f. produced for each solution was measured by the two proposed electrodes then the concentration of HyB was determined from the corresponding regression equations.

Calibration Graphs for Direct Potentiometry of Hyoscine Butylbromide

The conditioned electrodes were immersed in conjunction with the single junction calomel reference electrode in solutions of HyB in the range of 10^{-7} - 10^{-2} M. They were allowed to equilibrate whilst stirring and recording the e.m.f. readings within ± 1 mV.

The membrane sensors were washed between measurements with water. The mV-concentration profiles were plotted. The regression equations for the linear part of the curves were computed and used for subsequent determination of unknown concentrations of HyB.

Study of Experimental Conditions

Identification of Slope, Response Time and Lifetime of the Electrodes Studied. Electrochemical performance characteristics of the two studied HyB-selective electrodes (sensor №1 and №2) were evaluated according to IUPAC standards [26]. Sensors calibration was carried out by measuring the potential of 1×10^{-7} – 0.01 M drug solutions starting from low to high concentrations. The potentials were plotted as a function of drug concentrations. Sensor life span was examined by repeated monitoring of the slope of the drug calibration curve periodically. The detection limit was taken at the point of intersection of the extrapolated linear segment of the drug calibration graph.

The dynamic response times of the electrodes were tested for the concentrations 10^{-6} - 10^{-2} M HyB solutions. The sequence of measurements was from low to high concentrations. The time required for the electrodes to reach value within ± 2 mV from the final equilibrium potential after increasing HyB concentration level by ten folds was measured.

Effect of pH on the Electrode Response. The effect of pH on the potential values of the two electrode systems was studied over pH range 1-12 at 1-pH interval by immersing electrodes in 10^{-3} and 10^{-4} M HyB solutions. The pH was gradually increased or decreased by adding aliquots of diluted sodium hydroxide or hydrochloric acid solutions, respectively. The potential obtained at each pH was recorded.

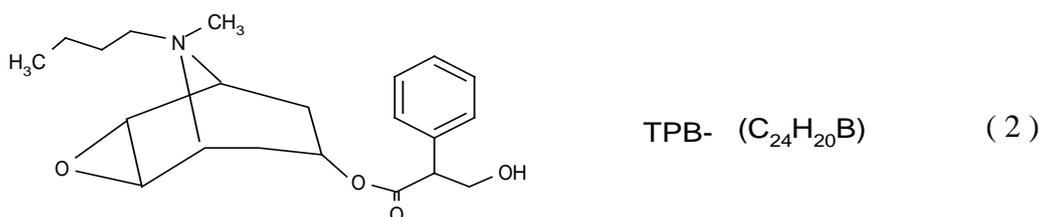
Effect of Interfering Compounds on the Electrode Selectivity. The response of the two studied electrodes was also examined in the presence of a number of other related substances. The potentiometric selectivity coefficients (K^{pot}_{ij}) were evaluated according to IUPAC guidelines using the separate solutions method [26,27], where potentials were measured for 0.001 M HyB solution and then for 10^{-3} M interfering solution, separately, then potentiometric selectivity coefficients were calculated using the following equation:

$$\log K^{pot}_{ij} = \frac{\Delta E_i - \Delta E_j}{S} \quad (1)$$

Results and Discussion

The strategy of the study presented herein was based on the fact that HyB is a quaternary ammonium salt that can act as a cation, suggesting the use of ion-exchangers of the anionic type (e.g. tetraphenyl borate) with the low solubility product and suitable grain size of the resultant precipitate.

HyB reacted with tetraphenyl borate to form stable 1:1 water insoluble ion association complex having the following suggested composition:



This ratio was confirmed by the elemental analysis (see Table I), and by the Nernstian response of both sensors being about 60 mV; i.e., typical for monovalent drugs [27].

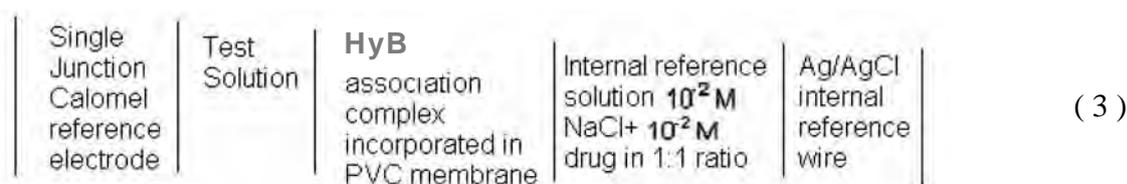
Table I. Elemental analysis of *Hyoscine Butylbromide*-tetraphenylborate complex.

Parameters	CHN-Analysis (in %)		
	Carbon	Hydrogen	Nitrogen
Calculated (in %)*	74.12	6.98	1.47
Found (in %)	75.52	7.15	1.39

*) Calculated according to 1:1 ratio

As found, PVC acts as regular support matrix, entrapping the ions sensed, but its use requires a plasticizer [28]. In this investigation, dibutylsebacate was chosen from diesters of dicarboxylic acids, and nitrophenyl octyl ether; the latter having been an example of the nitroaromatic group. They gave responses similar to each other with no noise but the membrane with nitrophenyl octyl ether was more sensitive as the corresponding calibration had ranged from 1×10^{-6} – $0.01 \text{ mol} \cdot \text{L}^{-1}$.

The electrochemical cell of the suggested membrane electrodes for the determination of HyB can be illustrated in the following diagram:



Electrochemical performance characteristics of the proposed sensors were evaluated according to the IUPAC recommendation data [26]; see Table II. It was found that the electrodes displayed constant and stable potential readings within 2 mV from day-to-day and the calibration slopes did not change by more than 2 mV per decade over a period of 1 month for the two sensors.

The response time of the electrodes were tested for concentrations of the drug from 1×10^{-6} – $0.01 \text{ mol} \cdot \text{L}^{-1}$. The measurements was characterized by a fast stable response within 20-30 seconds for concentrations less than $1 \times 10^{-4} \text{ M}$ and 10-20 seconds for concentrations higher than $1 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$.

Table II. Response characteristics of the two HyB-electrodes investigated

Slope (mV/decade)	-52.42	-54.57
Intercept (mV)	235.47	176.46
Correlation coefficient (r)	0.9991	0.9995
LOD (mol·L⁻¹)	2.5 x10 ⁻⁶	1.8 x10 ⁻⁷
Response time (s)	20-30	20-30
Working pH-range	6-10	7-10
Concentration range (mol·L⁻¹)	10 ⁻⁵ -10 ⁻²	10 ⁻⁶ -10 ⁻²
Life span (weeks)	6-8	6-8
Mean ± S.D.*	100.00 ± 0.96	99.92 ± 0.84

*) Results of three determinations, n = 3.

The effect of pH upon the electrode potential was investigated being found that the electrodes had given a linear range from pH 6-10 for sensor №1 and from pH 7-10 for sensor №2, respectively; see Figs. 2 and 3. Above and below this pH range, the potentials displayed by the electrodes were quite unstable ("noisy").

The potentiometric response of the two studied electrodes at the optimum pH were linear with constant slopes over a drug concentration range 1×10^{-5} – 0.01 M for sensor №1 and in the concentration range 1×10^{-6} – 0.01 mol·L⁻¹ for sensor №2 (see Figs. 4 and 5).

The linear regression equations were computed and found to be:

$$E = -52.42X + 235.47 \quad r = 0.9991 \text{ (sensor №1)} \quad (4a)$$

$$E = -54.57X + 176.46 \quad r = 0.9995 \text{ (sensor №2)} \quad (4b)$$

where "E" is the potential (in mV), "X" is the concentration (in mol·L⁻¹) and "r" is the correlation coefficient. The accuracy of the proposed membrane sensors for the quantification of blind samples of HyB was assessed by using the two sensors. The results showed average percentage recoveries of 100.00 ± 0.96 % and 99.92 ± 0.84 % for sensor №1 and 2, resp.

The performance of the two electrodes in the presence of PA was assessed. Selectivity coefficient values (K^{pot}_{ij}) were measured using a fixed concentration $0.001 \text{ mol}\cdot\text{L}^{-1}$ for the interfering species. The results obtained with both sensors developed are gathered in Table III, showing reasonable selectivity of the two sensors for HyB in presence of PA. (HyB was analyzed in mixtures with PA that had been prepared in different laboratories and satisfactory recoveries were obtained; see Table IV.)

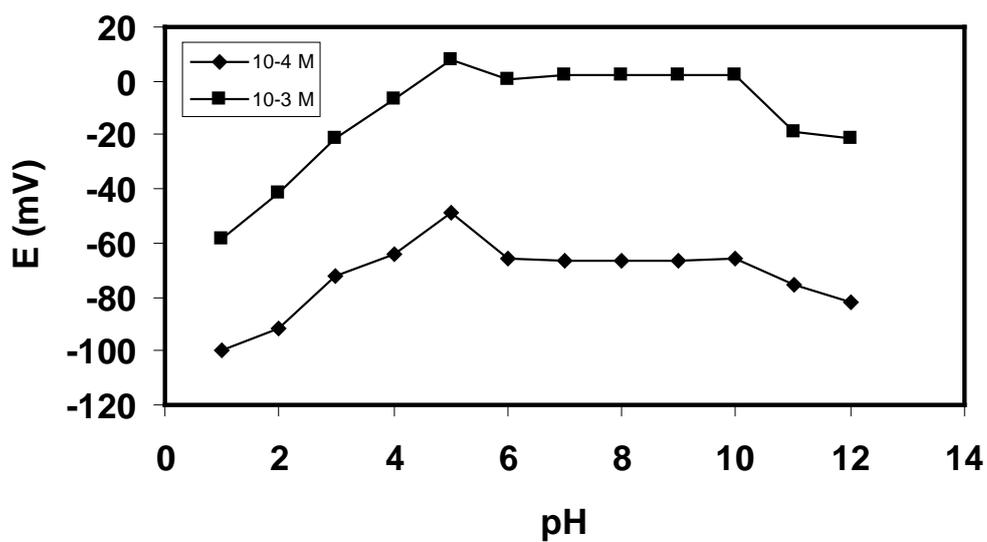


Figure 2. Effect of pH on the response of sensor № 1

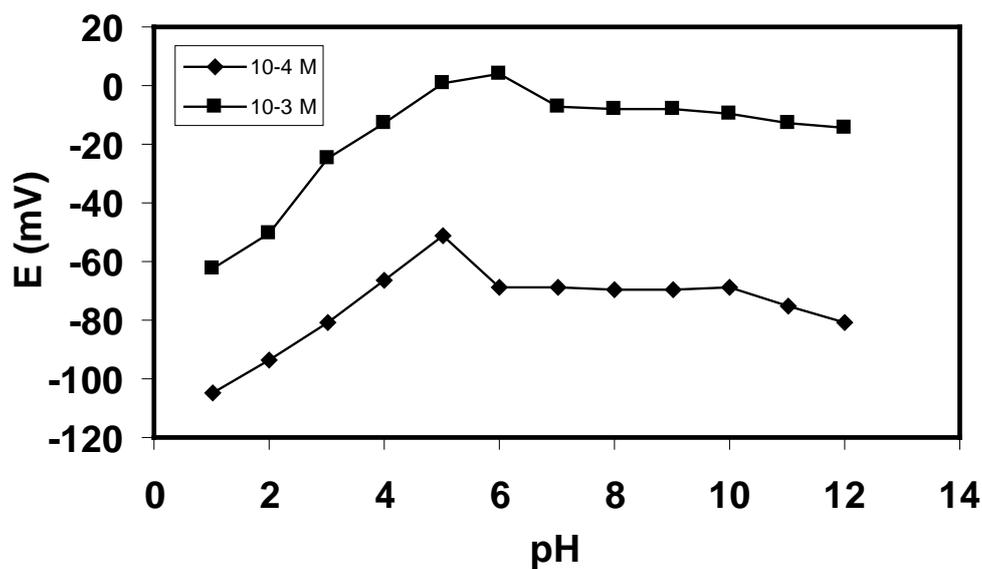


Figure 3. Effect of pH on the response of sensor № 2

Pharmaceutical additives did not show any interference. Thus, analysis was carried out without prior treatment or extraction. The two sensors were successfully used for the determination of HyB in *Buscopan plus* tablets and suppositories; see Table V.

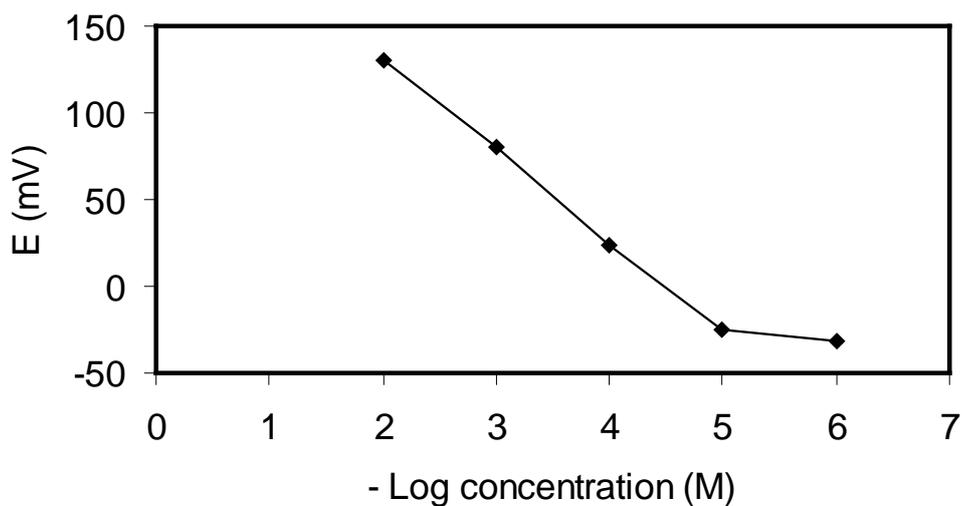


Figure 4. Profile of the potential (in mV) to the -log concentration of sensor № 1

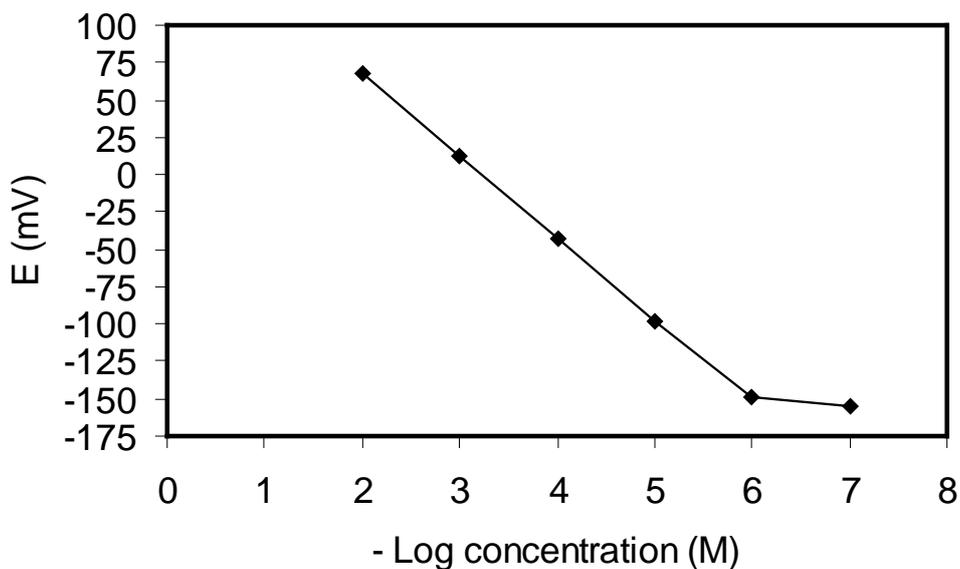


Figure 5. Profile of the potential (in mV) to the -log concentration of sensor № 2

On application to the biological fluids, plasma electrolyte did not show any interference. It has been found that the two electrodes gave stable results as revealed by high precision and accuracy of recoveries of the spiked plasma samples; see Table VI.

Table III. Potentiometric selectivity coefficients for the two electrodes tested

Interfering species	Selectivity coefficient (K_{ij}^{pot})	
	Sensor № 1	Sensor № 2
<u>Paracetamol</u>	5.89×10^{-3}	2.48×10^{-3}
Na ⁺	7.87×10^{-3}	3.01×10^{-3}
K ⁺	7.98×10^{-3}	2.77×10^{-3}
NH ₄ ⁺	5.35×10^{-3}	2.47×10^{-3}
Mg ²⁺	5.47×10^{-3}	2.63×10^{-3}
Ca ²⁺	6.43×10^{-3}	2.54×10^{-3}
Glucose	5.69×10^{-3}	2.37×10^{-3}
Lactose	5.58×10^{-3}	2.39×10^{-3}
Sucrose	5.22×10^{-3}	2.47×10^{-3}
Urea	6.07×10^{-3}	2.21×10^{-3}
<u>L-Phenyl alanin</u>	5.40×10^{-3}	2.07×10^{-3}

Table IV. Results of the analysis of *Hyoscine Butylbromide* (HyB) and *Paracetamol* (PA) in mixtures prepared in different laboratories

Ratio of HyB : PA	Recovery (in %) * of <i>Hyoscine Butylbromide</i>	
	Sensor № 1	Sensor № 2
1 : 1	100.40	100.41
1 : 0.5	100.68	99.59
1 : 2	100.67	99.86
Mean ± SD (%)	100.58 ± 0.16	99.95 ± 0.42

* Average of three determinations.

Finally, a statistical evaluation of the analysis of pure HyB by the proposed electrodes and the reported manufacturer method [29] showed that there is no significant difference between the developed and reported method in terms of accuracy and precision; see Table VII.

Table V: Quantitative determination of *Hyoscine Butylbromide* (HyB) in tablets and suppositories of *Buscopan plus*[®] by the proposed electrodes.

Pharmaceutical dosage forms	Recovery (% \pm S.D.) for HyB *	
	Sensor № 1	Sensor № 2
<i>Buscopan plus</i> ; tablets (batch № 205102)	98.57 \pm 0.47	98.32 \pm 0.35
<i>Buscopan plus</i> ; suppositories (batch № 1203115)	98.27 \pm 0.32	99.59 \pm 0.35

* Average of three determinations, n = 3.

Table VI. Determination of hyoscine butylbromide in spiked human plasma

Concentration (mol·L ⁻¹)	Recovery (% \pm S.D.) of HyB *	
	Sensor № 1	Sensor № 2
1 \times 10 ⁻³	98.34 \pm 1.57	99.01 \pm 0.89
1 \times 10 ⁻⁴	100.17 \pm 0.99	98.91 \pm 1.24

* Average of three determinations, n = 3.

Table VII. Statistical analysis of the results obtained by applying the electrode proposed and the colorimetric method [29] for analysis of HyB in the pure form.

Item (parameter)	Sensor № 1	Sensor № 2	Official method
Mean \pm S.D.	100.00 \pm 0.96	99.92 \pm 0.84	100.02 \pm 0.78
Number of analyses (n)	5	5	4
Variance	0.92	0.71	0.60
t ... 2.447*	0.03	0.17	--
F-test ... 9.28*	1.53	1.18	--

* The values between parentheses are the corresponding theoretical values of **t** and **F** at the 95 % confidence level.

Conclusions

It can be stated that the use of the proposed sensors offers the advantages of fast response, additional steps of a drug pretreatment or separation are eliminated and one can perform the direct determination of drugs in turbid and colored solutions when achieving remarkable detection limits. Thus, it seems that the two potentiometric sensors developed can be used in routine analysis; for instance, to determine the compound of interest — *Hyoscine Butyrbromide* (HyB) — in quality control laboratories. Last but not least, the results of this study confirm in its entirety the usefulness of inexpensive electrochemical measurements in modern pharmaceutical analysis; see e.g. [29] and refs. therein.

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