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Ion selective membrane electrodes for determination of benoxinate hydrochloride in pure form and in drug product

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ABSTRACT

This paper presents a comparative study between four sensors constructed to determine benoxinate hydrochloride (BX) in the presence of its hydrolysis induced degradation product using different ion association complexes and plasticizers. Precipitation based technique was used for sensors fabrication. The BX complexes with the cationic exchangers; BX-reinikate, BX- tetraphenylborate, BX-phosphotungestate, and BX- tetrakis were obtained in situ by soaking the PVC membranes in 1 x 10^{-2} BX solution. Dioctylphthalate and nitrophenyl octyl ether were used as solvent mediators. The proposed sensors showed fast, stable Nernstian responses across a relatively wide BX concentration range ($5x10^{-5}$ to 10^{-1}M) in the pH range of 4-6. The suggested sensors could be used for several weeks without any measurable change in sensitivity. They displayed good selectivity for BX in presence of its degradation product, common inorganic and organic species. The proposed sensors were successfully applied for the determination of BX in pure powder form and eye drops where good recoveries were obtained.

KEYWORDS

Benoxinate hydrochloride;
Reinikate;
Tetraphenylborate;
Phosphotungestate;
Tetrakis.

INTRODUCTION

Benoxinate HCl, 4-Amino-3-butoxybenzoic acid 2-(diethylamino) ethyl ester, monohydrochloride is a para-benzoic acid ester that is used as a local anaesthetic. It is used for topical anaesthesia of the eye for the fitting of contact lenses or removal of a foreign body from the corneal epithelium or for minor surgery. In 1% solutions it is also available for surface anaesthesia of the nose and throat[1].

The United States Pharmacopoeia determines BX solution in acetic acid and acetic anhydride by titration with 0.1N perchloric acid. Also it determines BX in ophthalmic solution by measuring its absorbance at 308 nm against 0.1 N HCl as a blank[2].

Several methods have been reported for the determination of BX including Spectrophotomety[3,4], atomic absorption[5], voltametry[6], capillary electrophoresis[7], gas chromatography[8,9] , and HPLC[10,11].

Only one membrane electrode was previously described for BX[12] where the authors prepared BX-sodium tetraphenyl borate as an ion pair association complex then incorporated it in PVC membrane using dioctyl phthalate as a plasticizer.

Full Paper

Ion selective electrodes (ISEs) based on material transport across a specific membrane are now widely used in the determination of drugs in pure and pharmaceutical dosage forms. The high selectivity of these electrodes imparts a great advantage over other techniques[13] as analytes in colored, turbid and viscous samples can be determined accurately without separation. Furthermore, they show rapid response to changes in concentration and are tolerant to small changes in pH. They are also simple and cheap to develop, setup and run[14]. Various reports have been published which highlight the important contribution of ion selective sensors for quantification of drugs[15,16].

The aim of this work was to develop simple easily prepared ion selective electrodes which can be used in routine quality control for the determination of BX in the presence of its hydrolysis induced degradation product in its drug substance and available pharmaceutical formulation without the need of preliminary extraction or separation steps.

EXPERIMENTAL

Apparatus

A Jenway digital ion analyzer model 3330 (Essex, UK) with Ag/AgCl double junction reference electrode no. 924017-LO3-Q11C was used for potential measurements. A pH glass electrode Jenway (Essex, UK) no. 924005-BO3-Q11C was used for pH adjustment.

Chemicals and reagents

Benoxinate hydrochloride, 100.04%, was obtained from Egyptian International Pharmaceutical Industries Co. (Cairo, Egypt). Benox® eye drops (4mg/ml, Egyptian International Pharmaceutical Industries Co. Cairo, Egypt) were used in this work.

All chemicals and solvents used were of analytical grade and water was bi-distilled. Nitrophenyl octyl ether (NPOE), dioctyl phthalate (DOP), dibutyl sybacate (DBS) were obtained from Sigma (St. Louis, USA)., ammonium reineckate (RN), sodium tetraphenylborate (TPB), sodium phosphotungestate tribasic hydrate (PT), tetrahydrofuran (THF), poly (vinyl chloride) (PVC) were obtained from BDH (Poole, England). Tetrakis (4-chlorophenyl)borate (TpClPB) was purchased from Aldrich (Steinheim, Germany). Potassium chloride, citric acid and dibasic sodium phosphate were obtained from Prolabo (Pennsylvania, USA). Citrate-Phosphate buffer (pH 4-6) was prepared by mixing different volumes of 0.1 mol L^{-1} citric acid and 0.2 mol L^{-1} dibasic sodium phosphate.

Standard solutions

BX working solutions (1×10^{-6} to 1×10^{-2}M) were prepared by serial dilutions from BX stock solution ($1x10^{-1}$M) using citrate-phosphate buffer pH 5 as a solvent.

Procedures

(a) Preparation of the membrane sensors

A portion of 10 mg of RN for sensor (1), TPB for sensor (2), PT for sensor (3), and TpClPB for sensor (4) was thoroughly mixed with 0.19 g PVC and 0.35 ml DOP in a 5 cm glass petri dish then dissolved in 5 ml THF. The petri dishes were covered with filter paper and left to stand overnight to allow solvent evaporation at room temperature. Master membranes with thickness of 0.1 mm were obtained and used for the construction of the electrodes.

(b) Preparation of the electrodes assemblies

From the prepared master membranes, a disk (≈ 5mm diameter) was cut using a cork borer and pasted using THF to an interchangeable PVC tip that was clipped into the end of the glassy electrode body. Equal volumes of 10^{-2} mol L^{-1} BX and 10^{-2} mol L^{-1} KCl were mixed and this solution was used as internal solution for electrodes. Ag/AgCl wire (1mm diameter) was immersed in the internal reference solution as an internal reference electrode.

The electrodes were conditioned by soaking in 1×10^{-2} mol L^{-1} BX solution for one day and were stored in distilled water when not in use.

(c) Electrodes calibration

The conditioned electrodes were calibrated by transferring 50 mL aliquots of solutions covering the concentration range of (1×10^{-6} to 1×10^{-2} mol L^{-1}) BX in citrate phosphate buffer pH 5, into a series of 100 ml beakers. The electrodes systems were immersed in each solution, in conjunction with a double junction Ag/AgCl reference electrode.

The electrodes were washed with distilled water

between measurements. The developed potentials were plotted versus negative logarithmic concentration of BX standard solutions. The regression equations of the obtained calibration plots were used for subsequent measurements of unknown samples.

(d) Determination of BX in its pharmaceutical formulation

A volume equivalent to 3.44 mg BX was accurately transferred to a 10-mL volumetric flask (to prepare 10^{-3} mol L^{-1} BX). The volume was completed to the mark with citrate phosphate buffer pH 5. The potentiometric measurements were performed using the proposed sensors in conjunction with the Ag/AgCl reference electrode, and the potential readings were compared to the calibration plots.

(e) Determination of BX in the presence of its alkaline degradate

BX degradation product was obtained by refluxing 1 gm BX with 100 mL 0.1 N sodium hydroxide for two hours. The resulting solution was tested for complete degradation by the thin layer chromatography technique using chloroform–methanol (95:5, v:v) as a mobile phase and detecting the spots at 254 nm. The pH of the degraded solution was then adjusted to 5.3 using 1 N hydrochloric acid to precipitate the degradation product. The precipitate was filtered, dried and protected from air and light[17]

Aliquots of standard drug solution (10^{-3} mol L^{-1}) were mixed with its degraded sample (10^{-3} mol L^{-1}) in different ratios. The emf values of these laboratory-prepared mixtures were recorded and results were compared with the calibration plot.

RESULTS AND DISCUSSION

Selective membranes in ion selective electrodes have shown both ion exchange and perm-selectivity for the sensor ion[18]. In this work, four ion selective membrane sensors were proposed for determination of BX either in its pure substance or drug product.

Membrane composition and response characteristics

Preparation of the proposed sensors originates from the fact that BX behaves as a cation in acid medium, this fact suggests the use of a cationic exchangers. The type of the ion exchanger affects the response of the sensor, therefore, four cationic exchangers, namely RN, TPB PT and TpClPB were used for the preparation of the membrane sensors as they form insoluble ion association complexes with suitable grain size with BX. The ratio of BX to the ion exchangers in the formed complexes was found to be 1:1 as proven by the obtained Nernestian slopes (about 60 mV/ decade) so BX acts as a monoionic species due to the presence of the tertiary amino group (Figure 1). The cationic exchangers were incorporated with a suitable solvent mediator in poly (vinyl chloride) matrix to produce plastic membranes which were used for constructing the electrodes. The complexes were formed in situ by soaking the prepared membranes in 1 x 10^{-2} BX solution.

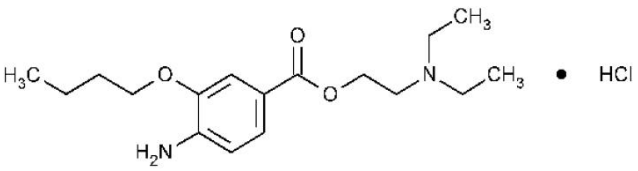

Figure 1 : Structure of benoxinate HCl.

The BX extraction into the membrane sensors was a result of the ion-pair tendency to exchange with BX cation. TABLE 1, shows the obtained slopes using different ion exchangers where near Nernestian slopes were obtained for all studied sensors.

TABLE 1 : Effect of the type of cationic exchangers and plasticizers on the slope and concentration range of BX.

Cationic exchanger	Plasticizer	Slope	Concentration range
TPB	DOP	55.8 ± 1.0	$1.0x10^{-5}$-$1.0x10^{-1}$
TPB	NPOE	53.0 ± 2.4	$1.0x10^{-5}$-$1.0x10^{-1}$
PT	DOP	59.4± 2.0	$5.0x10^{-5}$-$1.0x10^{-1}$
PT	NPOE	54.0 ± 3.0	$5.0x10^{-5}$-$1.0x10^{-1}$
RN	DOP	61.1 ± 1.0	$5.0x10^{-5}$-$1.0x10^{-1}$
RN	NPOE	56.5 ± 2.4	$5.0x10^{-5}$-$1.0x10^{-1}$
TpCIPB	DOP	58.6 ± 1.8	$5.0x10^{-5}$-$1.0x10^{-1}$
TpCIPB	NPOE	54.3 ± 2.3	$5.0x10^{-5}$-$1.0x10^{-1}$

The second factor that allows BX ions to be extracted from an aqueous solution into the membrane, as an organic phase, is the plasticizer. After the evaluation of two solvent mediators, namely NPOE and DOP, (as examples for plasticizers from diesters of dicarboxylic acids and nitroaromatic compounds respectively),

no significant change was obtained in the slopes by changing the solvent mediator. TABLE 1 shows that DOP, which was a less polar solvent mediator had a slight better response than NPOE that had a higher dielectric constant value leading to the extraction of polar ions, which had negative effects on the extraction of BX ion as a hydrophobic ion.

Based on the IUPAC recommendations[19] the response characteristics of the designed electrodes were assessed. TABLE 2 displays the results obtained over a period of two months for two different assemblies of each sensor. The calibration plots were presented in Figure 2. The slopes of the calibration plots were 61.1, 55.8, 59.4 and 58.6 mV/concentration decade for sensors 1, 2, 3 and 4 respectively. The deviation from the ideal Nernestian slope (60 mV/ decade), is due to the fact that the electrodes respond to activities of the drug rather than the concentration. The suggested electrodes displayed constant potential readings for day to day measurements, and the calibration slopes did not change by more than ±2 mV/decade over a period of 6 weeks. The detection limits of the sensors were estimated according to the IUPAC definition[19].

TABLE 2 : Validation of the response characteristics of the investigated electrodes.

Parameter	Sensor 1 BX-RN	Sensor 2 BX-TPB	Sensor 3 BX-PT	Sensor 4 BX-TpCIPB
Slope (mV/decade) [a]	61.1 ± 1.0	55.8 ± 1.0	59.4± 2.0	58.6 ± 1.8
Intercept (mV) [a]	209.6 ± 2.0	190.5 ± 1.0	228.8 ± 5.0	190.9 ± 4.0
Correlation coefficient (r^2)	0.997	0.995	0.994	0.994
Concentration range (M)	5.0x10-5-1.0x10-1	$1.0x10^{-5}$-$1.0x10^{-1}$	$5.0x10^{-5}$-$1.0x10^{-1}$	$5.0x10^{-5}$-$1.0x10^{-1}$
Response time (s)	10	15	15	10
Working pH range	4-6	4-6	4-6	4-6
Stability (weeks)	6	5	5	6
LOD	1.0x10-5	$5.0x10^{-6}$	$1.0x10^{-5}$	$1.0x10^{-5}$
Average accuracy [b] (% ± Standard deviation)	99.8 ± 1.3	99.9 ± 2.8	98.8± 3	99.8 ± 2.8
%Relative Standard deviation [c] (precision)				
Repeatability	2.0	1.7	2.0	2.4
Reproducibility	3.0	2.9	3.4	3.0
Robustness [d]	1.0	1.5	2.3	1.8
Robustness [e]	2.4	2.0	2.6	2.0
Ruggedness [f]	1.0	2.5	3.0	2.5

[a]Results of six determinations; [b]Average recovery % of five concentration levels (from 10^{-4} to 10^{-1}) each repeated three times; [c]Three concentration levels each repeated three times; [d]Relative standard deviation % of potential produced by 10^{-3} M solution (three times) at pH 4.5 instead of pH 5 (in phosphate buffer); [e]using DBS as plasticizer instead of DOP; [f]Relative standard deviation % of the potential produced by 10^{-3} M solution using Jenway 3505 digital ion analyzer instead of 3330.

A fast response time was recorded by increasing BX concentration by up to 10-fold. The required time for the sensors to reach values within ±1 mV of the final equilibrium potential was 10-15 s.

The optimum equilibration time for the electrodes, after soaking in 1.0 x 10^{-2} M BX, was 12 hours. After this time period, the electrodes generated stable potentials in contact with the BX solution. On soaking for a longer time the slopes decreased gradually and this may be attributed to the gradual leaching of the electroactive species into the bathing solution[12]. Therefore, when not in use for a long time, the electrodes should be kept dry.

To evaluate the precision of measurements, three concentrations within the linear concentration range (10^{-4}, 10^{-3} and 10^{-2} M solutions) of BX were chosen. Three solutions of each concentration were prepared and analyzed in triplicate (repeatability assay). This assay was repeated on three different days (reproducibility assay), (TABLE 2).

As for the robustness, the method demonstrated efficient stability when either the plasticizer or the ion exchangers were changed. Also, the wide range of pH (4-6) made the method robust. To study the method's

ruggedness, 10^{-3} M solution of BX was analyzed by the suggested electrodes using Jenway 3505 digital ion analyzer instead of 3330 Model. Results proved the ruggedness of the method upon changing the instrument (TABLE 2).

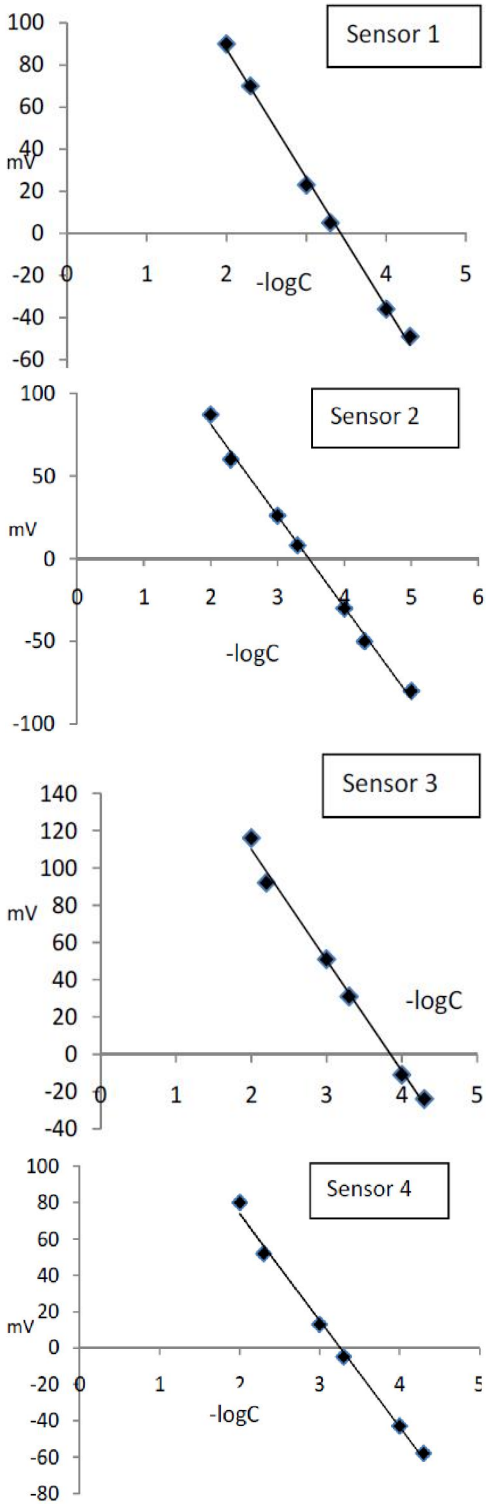

Figure 2 : Potentiometric profile of BX- sensors at pH 5.

pH effect on the electrodes responses

The potentiometric responses of the suggested electrodes were found sensitive to pH changes. Figure 3 showed a typical pH response curve for the prepared sensors over a pH range of 3-7, where the pH was adjusted by using Citrate-Phosphate buffer of different pH. The sensors responses were hardly affected by the pH change from 4 up to 6, i.e., in this pH range BX is completely ionized, dissociated and sensed, so all measurements were carried out at pH 5. The decrease in potential at pH > 6 was due to the gradual decrease in the concentration of the BX mono-cation due to the formation of the non-protonated amino group.

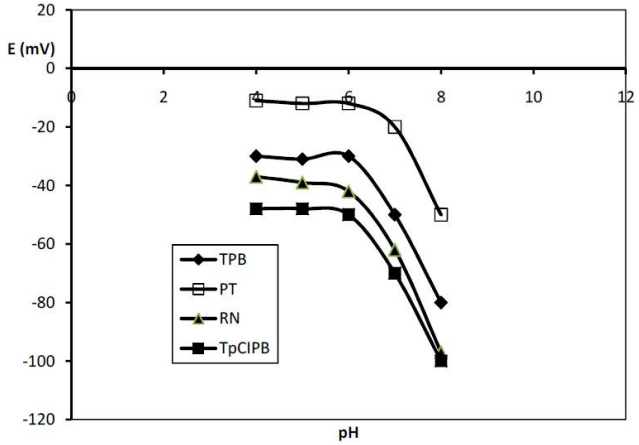

Figure 3 : Effect of pH on the response of the suggested sensors in 10^{-4} M BX.

Sensors selectivity

The selectivity of an ion-pair based membrane electrodes depend on the physico-chemical characteristics of the ion-exchange process at the membranes. TABLE 3 shows the potentiometric selectivity coefficients of the proposed sensors in the presence of a number of pharmaceutical additives commonly used in eye drops and other drugs prescribed for the eye. The selectivity coefficients were determined by the separate solution method and calculated from the rearranged Nicolsky Eisenman equation[19]:

$$-\log(K^{pot}_{\text{primary ion interferent}}) = \left[\frac{(E_{MD} - E_M)}{2.303RT/Z_{BX}F}\right] + \left[1 + \frac{Z_{BX}}{Z_M}\right]\log[BX]$$

Where E_{BX} and E_M are the potential readings recorded after exposing the electrode to the same concentration of the studied drug and the interferent, respectively, Z_{BX}

and Z_M are the charges on BX and the interfering ion, respectively and 2.303RT / Z_{BX}F represents the slope of the investigated sensor (mV / decade).

As it was obvious from TABLE 3, none of the tested interfering species had a significant influence on the potentiometric responses of the electrodes towards BX.

TABLE 3 : Potentiometric selectivity coefficients (*K*) for BX-RN sensor by separate solution method.

Interferent 10^{-3} M	Selectivity Coefficient* Sensor 1	Sensor 2	Sensor 3	Sensor 4
EDTA	$1.99x10^{-4}$	$9.5x10^{-5}$	$1.2x10^{-4}$	$2.0x10^{-4}$
Boric acid	$6.16x10^{-5}$	$3.2x10^{-5}$	$5.0x10^{-5}$	$7.9x10^{-5}$
NaCl	$4.78x10^{-5}$	$2.5x10^{-5}$	$2.5x10^{-5}$	$6.3x10^{-5}$
$ZnSO_4$	$6.91x10^{-5}$	$9.3x10^{-5}$	$7.9x10^{-5}$	$7.9x10^{-5}$
KCl	$8.9x10^{-5}$	$2.5x10^{-5}$	$3.3x10^{-5}$	$3.9x10^{-5}$
Glycine	$6.9x10^{-5}$	$2.6x10^{-5}$	$1.6x10^{-5}$	$1.2x10^{-5}$
tetrahydrazoline	$7.6x10^{-4}$	$8.5x10^{-5}$	$1.0x10^{-4}$	$7.9x10^{-5}$
Mepevacaine	$1.6x10^{-4}$	$8.5x10^{-5}$	$1.3x10^{-3}$	$2.0x10^{-4}$

***Average of three determinations**

Potentiometric determination of BX in pharmaceutical formulation

As none of the commonly used eye drops additives show significant interference with the determination of BX, the new proposed sensors were successfully applied for BX determination in eye drops without prior extraction as shown in TABLE 4. Results obtained prove the applicability of the method as demonstrated by the accurate and precise recovery percentages.

TABLE 4 : Statistical analysis between the results obtained for the determination of BX in Benox eye drops by the proposed sensors and those by the official method.

Item	Sensor 1	Sensor 2	Sensor 3	Sensor 4	official method[2]
Mean	100.1	101.3	100.8	100.5	100.2
S.D.	1.0	1.5	2	1.9	0.7
RSD%	1.0	1.5	2	1.9	0.7
Variance	1.0	2.3	4	3.6	0.5
n	3	3	3	3	7
F test	2.0 (4.88)	4.6 (8.9)	8.2 (8.9)	7.4 (8.9)	
Student's t test	0.2 (2.26)	1.7 (2.26)	0.74 (2.26)	0.4 (2.26)	

Figures between parenthesis are the corresponding tabulated values (P=0.05)

Potentiometric determination of BX in the presence of its alkaline degradate

BX was completely degraded when refluxed with 0.1 M NaOH. for two hours. Figure 4 shows the reported alkaline degradation of the drug[17]. TABLE 5 shows the results obtained upon analysis of synthetic mixtures containing different ratios of intact drug and degradation product. From the presented results it was obvious that the proposed sensors could be used for selective determination of intact drug in the presence of 30-40 % degradate.

TABLE 5 : Determination of BX in laboratory prepared mixtures containing different ratios of BX and its induced alkaline degradation product by the proposed sensors.

Ratio % Drug: Degradate[a]	Drug recovery % ± S.D.[b]			
	Sensor 1	Sensor 2	Sensor 3	Sensor 4
100: 0	98.6 ± 0.9	99.9 ± 0.8	100.4 ± 1.2	99.5 ± 1.2
90: 10	101.6 ± 1.4	98.9 ± 1.8	100.8 ± 0.8	99.8 ± 1.3
80: 20	100.6 ± 1.1	97.9 ± 1.5	100.3 ± 1.8	100.8 ± 0.9
70:30	98.1 ± 1.6	102.2 ± 1.8	100.9 ± 2.0	100.4 ± 0.8
60:40	117.8 ± 2.0	120.9 ± 1.7	107 ± 0.9	100.5 ± 1.7
50:50	150 ± 1.7	167 ± 2.3	159 ± 1.5	149 ± 2.1

[a]10 $^{-3}$ M solutions in beffer pH 5; [b]Average of three determinations

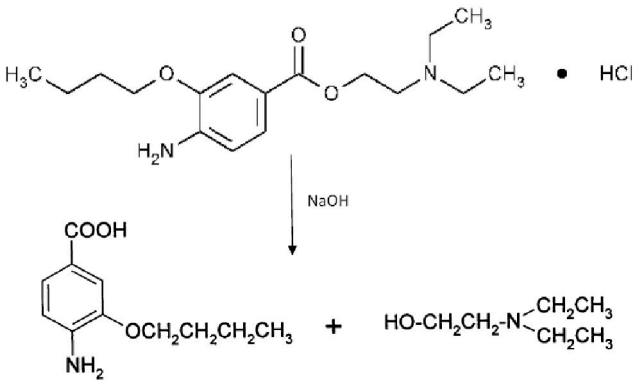

Figure 4 : Reported Alkaline degradation pathway of BX[6].

CONCLUSION

The four described sensors were simple and selective for the determination of BX in pure form, pharmaceutical preparation and in presence of degradation product. The ammonium reineckate sensor showed the best Nernestian slope while the sodium tetraphenylborate sensor had the best sensitivity. The sodium tetraphenylborate sensor described differed from that reported[12] that the sensor was prepared in situ and presented a stability indicating method as it was able to determine BX in presence of its degradate. The

proposed sensors offered advantages of fast response and elimination of drug pre-treatment or separation steps. They can therefore be used for routine analysis of BX in quality-control laboratories.

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