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FIA titrations of ephedrine in pharmaceutical formulations with a PVC tetraphenylborate tubular electrode*

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Abstract: A flow injection system for the titration of ephedrine in pharmaceutical products with potentiometric detection was developed. For this purpose a tetraphenylborate tubular electrode was constructed. The electrode was prepared without inner reference solution and with a PVC membrane based on tetrapentylammonium tetraphenylborate as ion exchanger and 2-nitrophenylphenyl ether as mediator solvent. Its operational characteristics were evaluated in a low dispersion manifold and compared with more conventionally shaped electrodes using the same sensor. In the pH range 2.5–11.5, the electrodes showed linear response between 3.8×10^{-6} and 0.1 M with a slope of −56.4 mV/log[BPh4]. Ephedrine determinations in pharmaceutical products were carried out in a single channel manifold with a mixing chamber incorporated and using the tubular electrode as detector. Recovery rates of 98.6 ± 2.5% were obtained in the analysis of tables, nasal drops and syrups with a sampling rate of about 60 h^{-1},

Keywords: *Ephedrine determination; flow injection analysis (FIA); tetraphenylborate ion selective electrode; pharmaceutical products.*

Introduction

Ephedrine is an alkaloid that stimulates both α- and β-adrenergic receptors. Chemical control of this product in pharmaceutical formulations is of great importance. Official pharmacopoeias [1, 2] recommend different methodologies according to the type of formulation to be analysed but usually determinations are carried out by titration in a non-aqueous media or LC. However, these methodologies require skilled personnel, are very time consuming and subject to interferences from drug excipients and different organic bases.

Sodium tetraphenylborate (NaBPh4) has been used extensively in the determination of various alkaloids. The end-point of the reaction of precipitation between sodium tetraphenylborate and the referred bases has been detected potentiometrically by using ion selective electrodes (ISE) [3–6]. However, earlier attempts of potentiometric titrations of ephedrine with sodium tetraphenylborate [5] showed unsatisfactory results. To overcome this problem titrations by the Gran's method using a liquid membrane electrode [7] were suggested. However the analyses are very time consuming and non-reproducible results are obtained. These aspects are related with the type of sensor system used and also with their mechanical instability arising from the use of an inner reference solution.

The elimination of the inner reference solution and the use of a convenient sensor allowed the construction of electrodes with tubular configuration and its use in simple and mechanically stable flow injection manifolds [8]. Moreover, flow injection analysis (FIA) with potentiometric detection is an advantageous alternative to present analytical methods which are still used in the control of different products namely of pharmaceutical interest. Standard FIA systems with potentiometric detectors using peak height measurements present several limiations including the inability to be used with classical titration chemistries [9]. The possibility of using FIA as

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a gradient technique where dispersion of the sample is controlled by an appropriate device and where a peak width is measured at a preset signal [9–12] reduces considerably the analytical time and consumption of the reagents and increases the sensitivity of each determination.

The aim of this paper is to construct a tetraphenylborate tubular electrode, without an inner reference solution and to use it in a FIA manifold for time-based titration of ephedrine in pharmaceutical products.

Experimental

Apparatus and electrodes

A Crison Model 2002 digital voltmeter (sensitivity ±0.1 mV) was used for the potentiometric measurements. In the FIA determinations the analytical signals were achieved by coupling the millivoltmeter with a Kipp & Zonen BD 111 recorder.

An Orion 90-02-00 double-junction reference electrode with 0.033 M sodium sulphate solution in the outer compartment was used. pH determinations were carried out by using a simple glass electrode Ingold, Ref. 10/402/3092.

In FIA manifolds the solutions were propelled by a Gilson Miniplus 2 peristaltic pump. A Rheodyne 5020 six-port rotary injection valve was used. The FIA manifold also comprises Omnifit Teflon tubing (0.8 mm i.d.), a grounding electrode, a support device for the reference electrode [14] and Gilson end-fittings and connectors. In ephedrine titrations a dilution chamber with incorporated magnetic stirrer [15] was also used.

Reagents and solutions

Analytical grade reagents and deionized water (specific conductivity <0.1 μS cm^{-1} were used in all experiments.

For assessing the working characteristics of the electrodes, a 0.033 M sodium sulphate solution was used as ionic strength adjuster. In the adjustment of ionic strength and pH of the samples for ephedrine determinations in pharmaceutical products, a phosphate buffer solution (pH 6.3; 0.1 M) was used. The ephedrine stock solution (10^{-2} M) was prepared from solid ephedrine hydrochloride by weighing and diluting in the buffer abovementioned. The less concentrated standards were obtained by subsequent dilution of the stock solution using the same buffer.

Standard sodium tetraphenylborate (NaBPh4) (10^{-1} M) was prepared from the correspondent solid, diluted with water, and standardized by potentiometric titration against thallium nitrate [16]. Less concentrated solutions used to perform the calibration curves of the electrodes were prepared from the most concentrated one by appropriate dilution with the same buffer or the ionic strength adjuster solution.

Membrane preparation and electrode assembly

The membranes of the tubular electrodes were prepared by mixing 25.0 ml of an aqueous solution of NaBPh4 0.1 M with 20.0 ml of 0.1 M tetrapentylammonium bromide in acetone. The acetone was then evaporated. The crystals formed (tetrapentylammonium tetraphenylborate (TPABPh4)) were washed with deionized water, filtered, and dried at room temperature.

Preparation of the sensor solution was carried out by dissolving 0.06 g of TPABPh4 into 2.42 g of solvent mediator (2-nitrophenylphenyl-ether (NPPE)). The membrane was prepared by addition of 0.40 ml of sensor solution to 0.18 g of PVC previously dissolved in tetrahydrofuran. The compositon of the membrane was approximately 1.7% (w/w) of TPABPh4, 67.3% (w/w) of NPPE, and 31.0% (w/w) of PVC.

Tubular electrodes were constructed as reported earlier [8] applying the sensor membrane directly on a solid support based in a mixture of a non-conductive resin with graphite powder.

Sample preparation

In liquid formulations (nasal drops and syrups) a volume equivalent to 2.5 mg of ephedrine hydrochloride was rigorously measured, and diluted to 25.00 ml with phosphate buffer.

For ephedrine determination in tablets or coated tablets, about 10 units were finely powdered and an amount equivalent to 12.6 mg of ephedrine hydrochloride was placed in a 25.00-ml volumetric flask and diluted with a phosphate buffer solution. FIA determinations were carried out directly on aliquots of this solution obtained after a five fold dilution. The results obtained with the developed methodology, were compared in order to assess their quality, with respect to recovery.

Results and Discussion

Electrode behaviour

Working characteristics of the tubular electrodes were assessed by using a low dispersion FIA manifold (Fig. 1a). The carrier stream was a solution of sulphate 0.033 M and NaBPh4 10^{-6} M to stabilize the baseline.

In order to reproduce, as possible, the conditions used in batch determinations for assessing the working characteristics of the conventionally shaped electrodes, an injection volume of 150 µl and a flow rate of 6.8 ml min^{-1} were used (Fig. 1a). These values allowed an analytical signal corresponding to about 98% of the stationary state signal.

Under these conditions, calibration curves were carried out for standard solutions of NaBPh4 whose ionic strength was adjusted to 0.1 M. Calibration curves were also carried out for phosphate buffer solution (pH 6.3), aiming its application to pharmaceutical formulations. Table 1 lists the mean values of the calibration parameters obtained with two tubular electrodes. For comparative purposes, the calibration data are included obtained during the evaluation of the corresponding conventionally shaped electrodes (Table 1) [13].

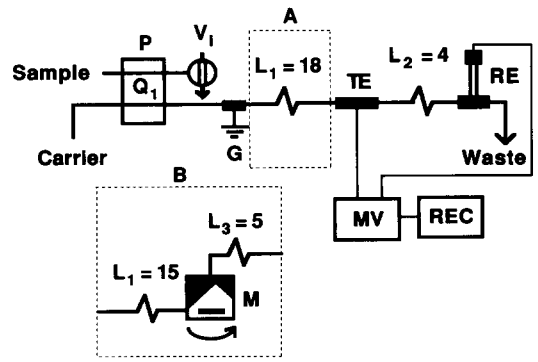

Figure 1
(A) Low dispersion flow injection manifold used for the tubular electrode evaluation/or (B) flow injection titration system: P — peristaltic pump; V_i-injection volume; TE — tubular electrode; RE — reference electrode; G — grounding electrode; MV — milivoltimeter; REC — recorder; M — well stirred mixing chamber; Q — flow-rate (6.8 ml min^{-1}); L_i-tube length cm^{-1} (0.8 mm i.d.); V_i for (A) manifold — 150 µl; V_i for (B) manifold — 80 µl; Carrier for (A) manifold — 0.033 M Na_2SO_4 and 10^{-6} M NaBP4; Carrier for (B) manifold — NaH_2PO_4/Na_2HPO_4 buffer solution and 10^{-4} M NaBP4.

With these experimental conditions, the operating characteristics of the tubular electrodes were very similar to those of conventionally shaped electrodes when evaluated by the batch method (Table 1). The slight difference in the lower limit of linear range (LLLR) of the tubular electrodes (Table 1) was a consequence of the residual concentration of the main ion used in the carrier during the evaluation of that parameter. High sampling rates (120 samples/h) for the analytical system were obtained. This is a consequence of the rapid response of the electrode due to its tubular configuration.

The influence of the pH in the tubular electrodes response was determined using the FIA manifold previously referred (Fig. 1a) with minor changes [17]. The determinations were carried out in 10^{-2} M and 10^{-4} M NaBPh4 solutions with ionic strength adjusted to 0.1 M. Under these conditions and within a pH range 2.5–11.5 units the variations in the detectors potential did not exceed 4 mV. This pH range was equivalent to that obtained with conventional electrodes [13].

The evaluation of the extent of interference of certain inorganic anions, which could be used as pH or ionic strength adjusters, was assessed by the separate solutions method [18]. Table 2 shows the values obtained for tubular and conventionally shaped electrodes.

The prepared tubular electrodes presented good general working characteristics, which

Table 1
Calibration parameters for tetraphenylborate tubular selective electrodes

Working characteristics	I*	II†	Conventional electrodes [13]
Slope (mV/log[BPh4])	−56.4 ± 0.9	−54.4 ± 0.9	−55.6 ± 0.4
Linear range (M)	3.8×10^{-6}–0.1	3.0×10^{-6}–0.1	2.5×10^{-6}–0.1
pH range‡	2.5–11.5	—	2.5–11.5
Reproducibility (mV day^{-1})	±1.5	±1.5	±1.0
Sampling rate (h^{-1})	120	120	—
Response time(s)	—	—	<20

* Values obtained in solutions with ionic strength adjusted to 0.1 M with Na_2SO_4.
† Values obtained in NaH_2PO_4/Na_2HPO_4 buffer solution (I = 0.1 M and pH 6.3).
‡ Results obtained in 10^{-2} M and 10^{-4} M NaBPh4 solutions

Table 2
Potentiometric selectivity coefficients for tetraphenylborate tubular electrodes ($\log K^{pot}$), at three concentration levels

	Tubular electrodes			Conventional electrodes [13]		
Interferent	10^{-4} M	10^{-3} M	10^{-2} M	10^{-4} M	10^{-3} M	10^{-2} M
Chloride	−2.1 ± 0.1	−3.2 ± 0.1	−4.2 ± 0.1	−1.9 ± 0.1	−2.8 ± 0.1	−3.7 ± 0.1
Nitrate	−2.0 ± 0.3	−3.1 ± 0.2	−4.1 ± 0.2	−1.4 ± 0.1	−2.4 ± 0.1	−3.4 ± 0.1
Perchlorate	−1.7 ± 0.3	−2.9 ± 0.3	−3.9 ± 0.3	−1.3 ± 0.1	−2.3 ± 0.1	−3.3 ± 0.1

justify their use in the determination of ephedrine in pharmaceutical formulations by FIA.

Ephedrine titrations in formulations

To achieve continuous flow titrations of ephedrine some changes were made on the manifold previously referred (Fig. 1b). A mixing chamber was used to create an exponential concentration gradient and to ensure a well-dispersed precipitate [19]. This mixing chamber had a 10-mm diameter base and a variable height, ensuring that the total volume (160 μl) could be selected. The magnetic bar rotated at around 320 rotations per min. Stirring was essential for good reproducibility of the results. Measurements of peak width (time interval) were made at a random value of potential (always the same in each batch) but near the baseline to provide the lowest possible inferior detection limit.

Samples of ephedrine standard solutions were inserted into a carrier stream of 10^{-4} M NaBPh4 prepared in phosphate buffer solution. This buffer solution was chosen according to the results obtained during the evaluation of the tubular electrodes in a low-dispersion manifold.

In order to carry out ephedrine determinations in different pharmaceutical products without pre-treatment of the samples, with good sensitivity (minimum concentration detected) and a high sampling rate, some parameters such as NaBPh4 concentration in the carrier, the flow rate and injection volume were studied and optimized.

The concentration of NaBPh4 in the carrier was the parameter that most influenced the titration because it determined the linear range and the detection limit. A 10^{-4} M NaBPh4 concentration was selected as a compromise between sensitivity and sampling rate. In fact a 10^{-5} M concentration produced determinations with a higher sensitivity though at a very low sampling rate since the peaks became considerably wider. On the other hand, a 10^{-3} M solution did not allow determination of low levels of ephedrine. The selected concentration did also ensure better reproducibility of the results.

The carrier flow rate was fixed at 6.8 ml min^{-1}. Lower flow rates produced an increase in the slope of the calibration curves but a significant decrease in sampling-rate.

The sample volume has little influence on the slope of the relation of log (ephedrine) versus time but large sample volumes cause a lower sample throughput rate. A volume of ca 80 μl was finally chosen.

The FIA system (Fig. 1b) was calibrated by injection (triplicate) of different ephedrine standards with concentrations varying from approximately 2×10^{-4} M to 2×10^{-3}M, prepared in NaH_2PO_4/Na_2HPO_4 (pH 6.3). Quantitative evaluation was based on peak-width measurement in mm (speed recorder 10 mm seg^{-1}) (Fig. 2). Under these conditions the calibration plot showed a straight line (r = 0.9994; N = 15) in the referred range and at a maximum sample rate of about 60 samples/h.

The reproducibility of the system was evaluated by making 21 injections of standard ephedrine solutions with concentrations of about 4×10^{-4} and 10^{-3} M. The relative standard deviation obtained was less than 1.5%.

Determination of ephedrine in pharmaceuticals

Application of the described FIA system was tested by analysing several pharmaceutical formulations with different compositions of common commercial occurrence in Portugal. Table 3 indicates the mean results and the corresponding standard deviation obtained with two electrodes on three independent preparations of each batch (mean of six values). To assess accuracy of the results recoveries were determined. The values obtained varied between ca 94 and 102%,

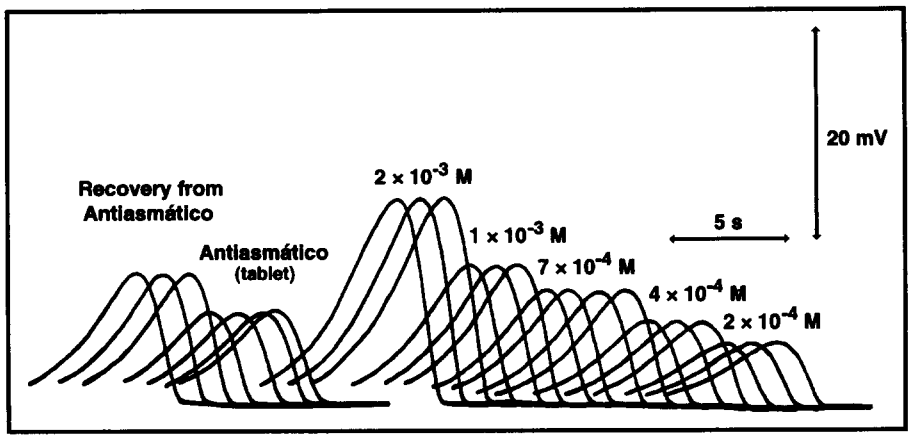

Figure 2
Typical recorder of analytical signals obtained by injection of different standard ephedrine solutions and a real sample (Antiasmático tablets) in the flow titration system.

Table 3
Results obtained by FIA titration in the analysis of different Portuguese commercial products containing ephedrine

Preparation*	Labelled (% w/w)	Obtained† (% w/w)	Recovery (%)
Antiasmático (tablet)	9.98	9.03 ± 0.31	98.9 ± 5.6
Argotone (nasal drop)	0.90	0.98 ± 0.06	98.0 ± 3.6
Nasocalma (nasal drop)	1.0	1.06 ± 0.03	94.3 ± 4.2
Efedril (syrup)	0.13	0.11 ± 0.01	98.4 ± 3.3
Congestan (syrup)	0.49	0.60 ± 0.04	101.5 ± 5.3
Codofosfol (syrup)	0.20	0.15 ± 0.02	97.7 ± 4.4
Constipal (coated tablet)	13.3	17.7 ± 2.9	101.7 ± 2.6

*Commercially available dosage forms with the commercial names written in Portuguese.
†Mean and standard deviation of three determinations with three electrodes ($n = 9$).

which corresponds to an average recovery of 98.6% and a mean standard deviation of 2.5%.

Conclusions

From the results obtained it is possible to conclude that tubular electrodes inserted in a FIA system of low dispersion have characteristics similar to those of conventional electrodes, using the same ionic extractor. Characteristics showed by the tubular detectors enabled the construction of a simple and mechanically stable FIA system which was then optimized for determination of ephedrine in pharmaceutical formulations of different chemical composition. This provides an alternative method to direct potentiometry or potentiometric titration in the determination of ephedrine in pharmaceutical formulations. The proposed method is less time consuming, economical regarding the equipment and reagents used and gave results with high accuracy and precision and is easy to be implemented in any routine laboratory work.

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