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Ion selective electrodes for penicillin-G based on Mn(III)TPP-Cl and their application in pharmaceutical formulations control by sequential injection analysis

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Abstract

The work describes the construction, evaluation and analytical application of ion selective electrodes sensitive to penicillin-G antibiotics for pharmaceutical products analysis. Different types of polymeric membranes based on PVC (poly(vinyl chloride)) and EVA (ethyl-vinyl-acetate), without internal reference solution, were prepared using 5,10,15,20-tetraphenylporphyrinate (TPP) manganese(III) (Mn(III)TPP-Cl) as electroactive material. Different additives such as tetra-*n*-octylammoniumbromide (cationic additive) and sodium tetraphenylborate (anionic additive) were incorporated into the membranes to evaluate their influence on electrodes performance. The comparison of the developed detectors was based on general analytical characteristics, selectivity and lifetime. To accomplish the analysis of real samples, two selective membranes composed of 33.0% (w/w) of PVC, 66.0% (w/w) of *o*-NPOE and 1.0% (w/w) of Mn(III)TPP-Cl (type A) and 33.0% (w/w) of PVC, 66.0% (w/w) of *o*-NPOE, 1.0% (w/w) of Mn(III)TPP-Cl and 10% mol (relative to the molar concentration of Mn(III)TPP-Cl) of sodium tetraphenylborate (type B) were used. Type A electrode presented a linear response between 2×10^{-5} and 10^{-1} mol l^{-1} for penicillin-G, a slope of about -59 mV dec^{-1} and a reproducibility of about ± 0.5 mV day^{-1}, while type B exhibited a linear response between 5×10^{-5} and 10^{-1} mol l^{-1} for penicillin-G, a slope of about -61 mV dec^{-1} and a reproducibility of about ± 0.3 mV day^{-1}. The potentiometric analysis of penicillin-G in pharmaceutical products was carried out by direct potentiometry and the results obtained were compared with those provided by the HPLC reference method.

These membranes (type A and type B) were used to prepare tubular electrodes that were coupled to a sequential injection system (SIA) and presented a linear range between 2×10^{-4} and 1×10^{-2} mol l^{-1} and slopes of -59.3 ± 0.8 and -57.3 ± 1.2 mV dec^{-1}, respectively. The tubular electrode constructed using type B membrane (type TB) was used to carry out the potentiometric analysis of penicillin-G in pharmaceutical formulations. The proposed procedure enabled relative errors between 0.1% and 1.2% (n = 4) and a sampling-rate of about 25 samples per h.

Keywords: Penicillin-G; Ion selective electrodes; SIA; Metalloporphyrins

1. Introduction

Penicillin-G or benzylpenicillin is an antibiotic produced by *Penicillium nonatum* mould, which includes in the structure a β-lactamic ring and a carboxylic group (Fig. 1A). Due to its large activity, this antibiotic is often used against both aerobic and anaerobic bacteria, being an important drug used in prevention and treatment of bacteria infections [1]. Its determination in pharmaceutical formulations is very important because penicillin-G is a relatively unstable drug and quality control parameters are generally required in indus-

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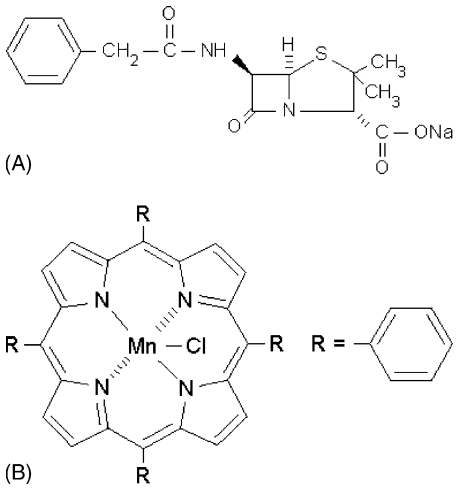

Fig. 1. (A) Penicillin-G chemical structure; and (B) Mn(III)TPP-Cl chemical structure.

try. Different methods are used for penicillin-G determination in pharmaceuticals, namely iodometric titrations and high-performance liquid chromatography (HPLC) with spectrophotometric detection [2], potentiometry and amperometry using biosensors [3,4], and even potentiometric ion selective electrodes based on anion exchangers [5]. Iodometric titrations are time-consuming and largely dependent on the operator, thus, providing less accurate results [2]. HPLC techniques although highly sensitive, are relatively expensive, demand fastidious sample preparation and are characterized by the use of toxic reagents. Potentiometric and amperometric determinations using biosensors, though being extremely sensitive, have several disadvantages such as short-term stability and high sensitivity to sample changes in temperature and pH. Ion selective electrodes based on quaternary ammonium compounds have been widely used in the determination of a series of anions [6], although providing electrodes with lack of selectivity towards other anionic species present as interferents, as a result of a potentiometric mechanism based exclusively on the ionic exchange of anions in membrane. Once the selective electrodes based on ion recognition electroactive species process involves both reversible and selective binding of ions, the design and synthesis of new ionophores for organic species becomes an important area of sensors research. Metalloporphyrins are a class of molecules that exhibit unique ionophore properties when incorporated into plasticized polymer membranes, and have recently been used in the preparation of ion selective electrodes, for the determination of salicylate [7,8], thiocyanate [9], chloride [10] and other organic and inorganic species [11]. The response mechanism of ion selective membranes based on these compounds is not only dependent on ionic exchange but also in the formation of ionophore–anion complexes provided by metallic central atom, allowing electrodes with improved selectivity towards different anions [12]. These characteristics of metalloporphyrins are exploited in this work on the construction of penicillin-G selective electrodes, using a manganese-porphyrin (Fig. 1B) as ionophore. To evaluate if the used porphyrin was acting as a charged or a neutral specie, different additives were added to the membrane composition, and electrode behaviour was observed and studied. Polymeric membranes based on PVC (poly(vinyl chloride)) and on ethylene–vinyl–acetate (EVA) were constructed using the same ionophore, to evaluate the behaviour of the metalloporphyrins when occluded in different polymeric support.

In order to attain more expeditious potentiometric determinations with less time and reagents consumption, the electrodes were coupled to a sequential injection analysis (SIA) system for the analytical control of penicillin-G in real samples. Besides improvement of general electrodes characteristics, continuous flow techniques such as sequential injection analysis, used in this work, provides an easier handling of samples and reagent solutions, higher sampling rates and cleaner determination procedures.

2. Experimental

2.1. Reagents and solutions

Analytical grade chemicals were used without any additional purification. All solutions were prepared by carefully weighing the corresponding solid followed by dilution with distilled and deionised water (conductivity < 0.1 $\mu S\,cm^{-1}$).

The reagents employed in the preparation of sensor membranes were as follows: manganese(III)tetraphenylporphyrin chloride (Mn(III)TPP-Cl, Aldrich) as ionophore, *o*-nitrophenyl octylether (*o*-NPOE, Fluka) as plasticizer, high molecular weight poly(vinyl chloride) (PVC, Fluka) and ethyl-vinyl-acetate (EVA, Bayer) as polymeric supports and tetra-*n*-octylammoniumbromide (TOABr, Fluka)/sodium tetraphenylborate (NaTPB, Fluka) as additives. Tetrahydrofuran (THF, Riedel-de-Haen) and chloroform ($CHCl_3$, Merck) were the solvents employed for membrane preparation.

Benzylpenicillin sodium salt (penicillin-G·Na, Sigma) was used in the preparation of standard solutions. A stock solution of this salt was prepared daily by weighing the solid and dissolving it in a 0.05 mol l^{-1} NaH_2PO_4 (Merck) solution as ionic strength adjuster. Less concentrated solutions were obtained after dilution of the previous one on the same solution, which was also used as electrolyte when carrying out the evaluation of the response characteristics of the electrodes and as carrier solution, to accomplish pH and ionic strength adjustment of the injected samples.

In real samples, determined by HPLC method, the mobile phase was constituted by KH_2PO_4 (Merck) (pH 3.5), deionised water and methanol (Merck) in a proportion of 10:54:36 (v/v/v). This eluent was also used for the preparation of penicillin-G standard solutions and phenylacetic acid (Sigma) internal standard solutions (0.2 mg ml^{-1}).

For samples preparation, an amount of powder was weighed or a volume of suspension measured, which was

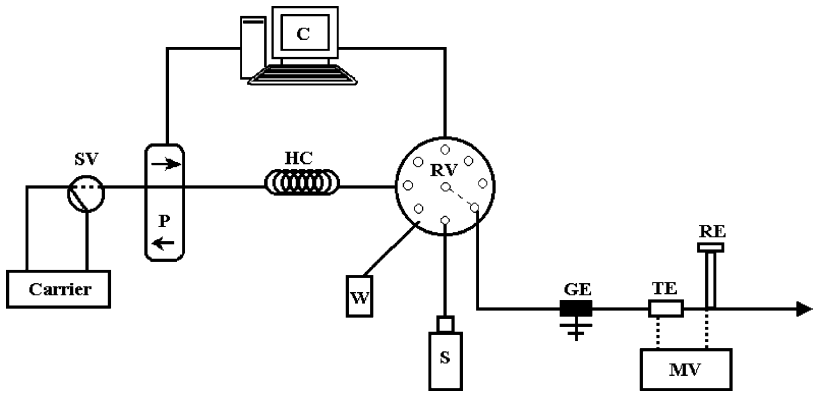

Fig. 2. Sequential injection system: solenoid valve (SV); peristaltic pump (P); rotatory valve (RV); sample or standard solutions (S); holding coil (HC); waste (W); grounding electrode (GE); penicillin-G selective electrode (TE); reference electrode (RE); computer (C); and decimillivoltimeter (MV).

then dissolved in NaH_2PO_4 0.05 mol l^{-1}, in order to obtain a solution of penicillin-G with the unit content that fitted in the linear range of the developed electrodes.

For the reference method, based on HPLC technique [2], different sample preparations were adopted. Samples were prepared in order to have 50 mg of penicillin-G in 50 ml of eluent, to compare with the standard solution, which has the same quantity. All solutions were prepared just before use, in order to avoid penicillin-G degradation.

2.2. Apparatus and electrodes

A Crison 2002 pH potentiometer (sensitivity: ±0.1 mV) coupled to an Orion 605 electrode switcher was used for measuring the potential differences between Orion 90-02-00 double junction AgCl/Ag reference electrode and the indicator electrode.

The pH values of all solutions and the operational pH range characteristics of the electrodes were determined with a Phillips GAH 110 glass electrode.

A schematic view of the proposed SIA system is depicted in Fig. 2. It comprised a Minipuls 3 Gilson (Villiers-le-Bel, France) peristaltic pump with a PVC pumping tube (i.d., 1.65 mm) of the same brand, a multiposition 8-port fluid selecting valve (RV) from Valco Instruments (Houston, USA), model Cheminert™ C15-3118E and a Crison 2002 pH potentiometer to which a 90-02-00 Orion AgCl/Ag reference electrode (0.05 mol l^{-1} NaH_2PO_4 solution in the external compartment) and a tubular detector were connected. This tubular penicillin-G detector was constructed following the previously described methodology [13] and was also connected to port 2 of RV with an 80 cm length tube. All tubular paths were made of 0.8 mm i.d. PTFE tubing. The holding coil (HC) between the peristaltic pump and the rotary valve was 400 cm long and was coiled over a plastic net. Some home made devices, such as joint pieces, grounding electrode supports for tubular and reference electrodes, as described elsewhere [14], were also used.

In order to achieve maximum reproducibility of the aspirated and forward volumes, a three-way NResearch 161 T031 (Stow, USA) solenoid valve was placed between the carrier channel and the peristaltic pump. This valve was activated by establishing electric contact on an electronic switch placed in a defined position of the rotating head of the peristaltic pump [15].

The rotation speed of the peristaltic pump (P), the rotor position of the eight-port valve (RV) and the solenoid valve (SV) on/off switching were controlled through an Advantech PCL-711B interface card by running software written in Quick Basic programming language.

A chromatographic system (Merck Hitachi) composed of a Rheodyne model 7100 pump, a Rheodyne 7725i injector (20 μl loop) and a RP 18 chromatographic column (250 mm × 4 mm) packed with 10 μm Lichrosorb particles was used. As detector, a diode array system (model 7455) was utilized while the data was processed by incorporated software (model D7000).

2.3. Membrane preparation and electrode construction

The potentiometric sensor solutions were prepared according to the procedure described [16] by dissolving metalloporphyrin-Mn(III)TPP-Cl into the plasticizer solvent (*o*-NPOE). A solution of PVC (in tetrahydrofuran) or EVA (in chloroform) was added to the previous one in order to obtain the membranes. Some membranes were also prepared by incorporation of anionic or cationic additives to evaluate their influence on the selectivity characteristics of the electrodes. As cationic additive was selected tetra-*n*-octylammoniumbromide and sodium tetraphenylborate was chosen as anionic additive. Thus, polymeric membranes were composed of 1% (w/w) Mn(III)TPP-Cl, 66% (w/w) *o*-NPOE and 33% (w/w) PVC/EVA. Particularly, in the case of PVC supports, in some experiments, TOABr was also added as well as NaTPB in different proportions (see Table 1).

The selective membranes were applied dropwise over the graphite conductive support [16] and membranes were

Table 1
Membrane composition (%, w/w) of penicillin-G conventionally-shaped and tubular electrodes (TA and TB) constructed

Types	A/TA	B/TB	C	D	E	F
Mn(III)TPP-Cl	1	1	1	1	1	1
o-NPOE	66	66	66	66	66	66
NaTPB	–	10% (mol)[a]	20% (mol)[a]	–	–	–
TOABr	–	–	–	10% (mol)[a]	20% (mol)[a]	–
PVC	33.0	33.0	33.0	33.0	33.0	–
EVA	–	–	–	–	–	33.0

[a] Percentage given: molar amount of TOABr or NaTPB relative to the total ionophore concentration in the membrane.

dried at room temperature over about 12 h and soaked in 0.01 mol l^{-1} solution of penicillin-G for 2 h before use. The electrodes remained in contact with the same solution when not in use.

Tubular detectors were developed afterwards according to [13] using the membranes composition that presented the best characteristics observed for the conventional ones. In this case, the resulting tubular detectors were conditioned after coupling to the system and flowing through the above-mentioned solution at a flow rate of 0.1 ml min^{-1} for the same period of time.

2.4. Sequential injection system

Initially the holding coil (HC) and the conveyance paths from the eight-port RV were all filled up with carrier solution (Fig. 2). Then by selecting port 6 of the RV and positioning the peristaltic pump (P) in the propulsion mode carrier was pumped until a detector stable signal was achieved. Then, previous evaluation of hydrodynamic variables such as flow-rates and injection-volumes were performed in order to guarantee analytical signals with intensity independent of injection-volumes and maximum sampling rate [15]. Therefore, when optimum flow conditions were achieved, the assessment of the tubular electrode behaviour as well as real sample analysis was performed. On these conditions, calibration curves were obtained by sequential aspirations of standard solutions of penicillin-G from port 5 of the RV (140 μl) into the holding coil (HC) of the sequential injection manifold. Then, port 6 was selected and the direction of the carrier stream was reversed each injected solution being propelled towards the detector during 100 s at a 3.8 ml min^{-1} flow-rate. Solutions of penicillin-G with concentrations of 1×10^{-4}, 3×10^{-4}, 5×10^{-4}, 8×10^{-4} and 1×10^{-3} mol l^{-1} were used as calibrating solutions, in which sample concentration fitted in. Therefore, following the same procedure, 140 μl of sample was aspirated and propelled towards the detector and the change of potential was registered.

2.5. Sample preparation

Formulations of penicillin-G, commercialised in Portugal for human use and for veterinary applications, were analysed. Different formulations were determined, namely: Lentocilin® S2400 (penicillin G-benzathine); Penadur® L-A (penicillin G-benzathine); Prevacilina® forte (penicillin G-clemizol, penicillin G-Na); Prevacilina® mega (penicillin G-clemizol, penicillin G-Na); Atralcilina® Vet 10 (penicillin G-procaine); Atralmicina® Vet (penicillin G-procaine) and Atralmicina® Vet 12 + 12 (penicillin G-procaine). All pharmaceutical products presented their antibiotic content in penicillin G units and for that reason results will be presented in these units. Depending on the solubilities of the different penicillin G salts presented in the pharmaceutical formulations tested (benzathine, procaine, clemizol or potassium) different salt concentrations were prepared.

For samples preparation, an amount of powder was weighed or a volume of suspension measured, which was then dissolved in NaH_2PO_4 0.05 M, in order to obtain a solution of penicillin-G with the unit content that fitted in the linear range of the developed electrodes.

For the reference method based on HPLC technique [2], different sample preparations were adopted. Samples were prepared in order to have 82,900 units of penicillin-G, to compare with the standard solution, which has the same quantity. All solutions were prepared just before use, in order to avoid penicillin G degradation.

3. Results and discussion

3.1. Evaluation of conventionally shaped electrodes characteristics

Metalloporphyrins behaviour varies according to the central metal (M) that is inserted in the porphyrin ring. That way, a M(II) porphyrin is expected to act as neutral carrier, because the introduction of anionic sites in the membrane provides a cationic potentiometric answer, while the addition of cationic species enables electrodes with a Nernstian slope toward anions [17]. On the other hand, M(IV) porphyrins exhibit a charged mechanism, and it was shown that the addition of lipophilic anionic sites to Sn(IV)TPP-Cl_2 membranes sensitive to salicylate [18] was required for analytically useful responses toward anions. A M(III) center within a given porphyrin structure will enable the ionophore to act as either a neutral or a charged carrier [18] depending on the number of negatively charged ligands on the metal center. When two anionic ligands are present in the metallic center, the metalloporphyrin generally acts as neutral; if in

the coordination site is present a single anionic specie, the other being a neutral molecule like water, the metalloporphyrin acts as charged. Therefore, the optimum mechanism of these membranes can only be evaluated experimentally. Thus, membrane sensors incorporating plasticized PVC and EVA as polymeric matrix membranes with Mn(III)TPP-Cl ionophore were prepared with different additives (Table 1) and their potentiometric characteristics were evaluated. A final value of 1% (w/w) of Mn(III)TPP-Cl was selected for the preparation of all membranes once it was demonstrated that an increase of ionophore in membrane provided electrodes with slightly higher slopes and lower limits of linear response (LLLR) but with less reproducibility [19]. It can be observed in Table 2, that the developed membranes for penicillin-G determination, based on Mn(III)TPP-Cl, exhibit different behaviour relatively to PLD, LLLR and reproducibility. Type F membrane prepared with EVA as polymer presented a slope of $-55.1\,\mathrm{mV\,dec^{-1}}$, as well as a lower sensitivity (higher PLD and LLLR) relatively to the PVC membrane with similar membrane composition (type A). It can be observed that the type of polymer used to occlude the sensor solution plays an important role on the potentiometric response of the electrodes, especially when it concerns to slope values and interference level, and that was also demonstrated by [20] for the development of ISE sensitive to tetracycline antibiotics based on sodium tetraphenylborate.

For type A membranes (without additive), PLD and LLLR values are relatively lower than those presented for membrane types B and C (with NaTPB), but reproducibility seems to increase by the presence of increasing amounts of anionic additive. PVC membranes containing TOABr (types D and E) evidenced a super-Nernstian potentiometric response (slopes of -69.1 and $-73.4\,\mathrm{mV\,dec^{-1}}$, respectively), and characteristic calibration curves (Fig. 3), in which linearity is only present for concentrations above 2.4×10^{-4} and $9.1 \times 10^{-4}\,\mathrm{mol\,l^{-1}}$, respectively. Besides, the reproducibility values presented by these units are considerably worse than those registered for all the other electrodes. From this comparative evaluation two different conclusions were made possible. First, the negative nature of the slope did not change with the addition of both cationic and anionic additives thus evidencing a charged mechanism of response. Second, the use of oppositely charged additives, such as TOABr, favours the presence in the membrane of uncomplexed ligands in order to preserve the electro neutrality [21] and this favours dimerization of the ionophore. However, LLLR and PLD became worse and interferences also increased, as shown in previous work [19]. For those reasons, these membranes were considered inadequate for direct potentiometry, especially for continuous flow conditions, although their analytical characterization was useful for the assessment of the response mechanism of Mn(III)TPP-Cl.

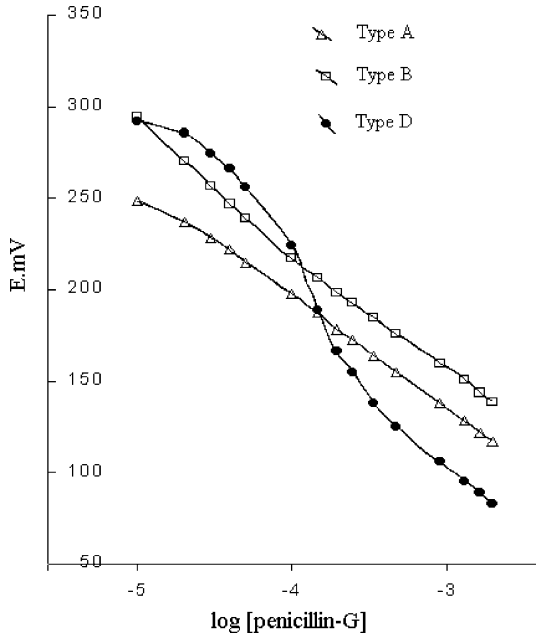

Fig. 3. Calibration curves of membranes type A (without additive), B (with anionic additive) and D (with cationic additive); the amount of additive is equal in type B and D electrodes.

Types A and B units' slopes are near-nernstian and that sensitivity makes them suitable for potentiometric determination of penicillin-G in real samples.

Table 2
General working characteristics of the constructed ISE selective to the penicillin-G

Electrode type	Slope ($\mathrm{mV\,dec^{-1}}$)	LLLR ($\mathrm{mol\,l^{-1}}$)	PLD ($\mathrm{mol\,l^{-1}}$)	pH range	Reproducibility ($\mathrm{mV\,day^{-1}}$)
Conventionally-shaped electrodes					
A	-59.1 ± 1.1	2.0×10^{-5}	9.9×10^{-6}	3.5–9.0	±0.5
B	-61.1 ± 0.6	4.9×10^{-5}	3.0×10^{-5}	3.2–7.0	±0.3
C	-62.9 ± 0.7	4.0×10^{-5}	2.9×10^{-5}	3.6–6.5	±0.3
D	-69.1 ± 2.5	2.4×10^{-4}	1.9×10^{-4}	a	±2.1
E	-73.4 ± 2.8	9.1×10^{-4}	2.4×10^{-4}	a	±2.6
F	-55.1 ± 1.0	9.9×10^{-5}	5.0×10^{-5}	4.7–7.5	±0.6
Tubular electrodes					
TA	-59.3 ± 0.8	2.0×10^{-4}	1.5×10^{-4}	a	±0.3
TB	-57.3 ± 1.2	2.0×10^{-4}	1.5×10^{-4}	a	±0.2

(a) This parameter was not evaluated.

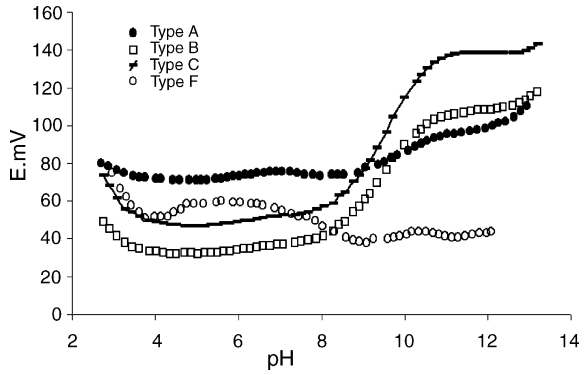

Fig. 4. pH profile for types A, B, C and F penicillin-G electrodes in solutions of 10^{-2} mol l^{-1} of penicillin-G.

It has been reported previously that ionophores based on transition metal complexes, such as metalloporphyrins, can exhibit significant pH sensitivity and cannot bind anion themselves at pH values higher than four due to their neutrality [22]. Hence, the influence of pH on the potential values of electrodes was evaluated for 0.01 mol l^{-1} solutions of penicillin-G (Table 2). Typical results for the electrodes based on Mn(III)TPP-Cl are shown in Fig. 4. The pH was changed by adding concentrated sulphuric acid, or concentrated potassium hydroxide solution. As shown in Table 2, the operational pH range decreases for electrodes prepared with NaTPB, relatively to the one without additive, especially for higher values of pH. Probably the presence of anionic additives favours the coordination of OH^- anion, instead of the carboxylate anion, such as penicillin-G, with the metal center. In fact, OH^- and penicillin-G^- ions coordinate competitively with the metal center of the ionophore. It can be seen in Fig. 4, that the response of the electrode is hardly affected by pH changes in the range of 3.5–7.0. As the pH of solution increases, OH^- becomes the primary ion and, consequently, the potentiometric response of the electrode for penicillin-G^- ion may deviate from linearity. Therefore, better responses to penicillin-G are expected at low concentrations of OH^- being adequate to work at an acidic region of the pH potential response curve. The working pH chosen for all measurement conditions was 4.5, which guaranteed, according to the penicillin-G pK_a, that all analyte was present as anionic specie. For type F electrodes, formulated with EVA as polymeric membrane, a decrease in the operational pH range was observed, especially for low pH values, which also limits its analytical application.

One of the most important characteristics for ISE's is its relative response for the primary ion over other ions present in solution, which is expressed in terms of potentiometric selectivity coefficients. The aim was the evaluation of potentiometric selectivity of membranes containing no additive (type A), different types and amounts of additives (types B, C, D and E), as well as different polymeric membranes (types A and F). The logarithmic potentiometric selectivity coefficients, log $K_{\text{pen-G}}$, of some common inorganic and organic anions were determined by the separated solutions method [23] (Table 3). For anion selective electrodes, it is well known that positively charged ionophores need lipophylic sites with the same charge of the primary ion, to improve characteristics as selectivity and reproducibility [17]. It is assumed that the presence of a small amount of anionic species can stabilize the membrane, by complexation with the charged ionophore. Only by the presence of strong ligands, equilibrium will be shifted towards the free carrier, enabling the potentiometric answer [24]. When no additives are present, the carrier has the possibility to react with strong ligands, but also with less strong species, being less selective than the membranes with anionic additives. An interesting behaviour can be observed by the introduction of NaTPB in the constructed electrodes. Comparing electrode types A and B (Fig. 5), it

Table 3
Potentiometric selectivity coefficients (log K^{pot}) for two different concentrations (3×10^{-4} and 3×10^{-3} mol l^{-1})

Interferent vs. type of electrode	A	B	C	F
Sulphate	−1.20 ± 0.04	−1.67 ± 0.03	−1.65 ± 0.06	−1.04 ± 0.05
	−2.25 ± 0.06	−2.64 ± 0.02	−2.65 ± 0.03	−2.14 ± 0.05
Bicarbonate	−1.14 ± 0.03	−1.65 ± 0.05	−1.54 ± 0.05	−0.66 ± 0.04
	−1.75 ± 0.08	−2.75 ± 0.03	−2.45 ± 0.08	−1.17 ± 0.05
Chloride	−1.08 ± 0.04	−0.98 ± 0.06	−1.04 ± 0.03	−0.83 ± 0.02
	−1.60 ± 0.06	−1.33 ± 0.07	−1.29 ± 0.04	−1.46 ± 0.01
Nitrate	−1.00 ± 0.03	−1.10 ± 0.04	−1.35 ± 0.03	−0.42 ± 0.08
	−1.52 ± 0.03	−1.42 ± 0.03	−1.75 ± 0.02	−0.69 ± 0.09
Nitrite	−0.95 ± 0.02	−0.84 ± 0.03	−0.87 ± 0.01	−0.61 ± 0.07
	−1.26 ± 0.03	−0.99 ± 0.03	−0.88 ± 0.01	−1.07 ± 0.04
Ampicillin	−0.90 ± 0.02	−0.95 ± 0.03	−0.86 ± 0.03	−0.77 ± 0.02
	−1.34 ± 0.05	−1.29 ± 0.04	−1.08 ± 0.02	−1.18 ± 0.02
Iodide	−0.17 ± 0.03	−0.24 ± 0.01	−0.42 ± 0.02	0.56 ± 0.08
	−0.24 ± 0.03	−0.32 ± 0.01	−0.49 ± 0.02	0.72 ± 0.08
Salicylate	1.18 ± 0.05	1.38 ± 0.03	1.42 ± 0.03	1.36 ± 0.05
	1.18 ± 0.10	1.50 ± 0.09	1.52 ± 0.07	1.67 ± 0.08
Thiocyanate	1.52 ± 0.04	1.96 ± 0.03	1.99 ± 0.05	1.81 ± 0.03
	1.49 ± 0.04	2.09 ± 0.08	2.09 ± 0.09	1.95 ± 0.10

Stated values represent the mean and standard deviation obtained after two determinations performed with two electrodes.

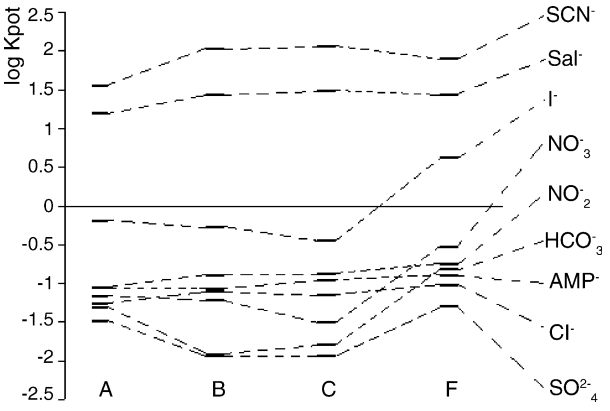

Fig. 5. Selectivity coefficients of penicillin-G electrode types A, B, C and F to a concentration of 1×10^{-4} mol l^{-1}.

Table 4
Differences between electrodes based on Mn(III)TPP-Cl and anion exchangers (TOMAC and TBHDPB)

	This work	Reference [5][a]	Reference [5][b]
LLLP (mol l^{-1})	4.9×10^{-5}	6.3×10^{-5}	2.5×10^{-4}
PLD (mol l^{-1})	3.0×10^{-5}	1.0×10^{-5}	1.2×10^{-4}
Slope (mV dec^{-1})	-61.1 ± 0.6	-49 ± 5	-57 ± 3
Response-time (s)	30	100	100
Interferents (log K^{pot})	<1.5	<2.7	<2.6
Salicylate	<2.1	<3.5	<3.1
Thiocyanate	<−1.42	<1.2	<1.2
Nitrate	<−0.32	<2.8	<2.8
Iodide			

[a] Based on TOMAC (trioctylmethylammonium chloride).
[b] Based on TBHDPB (tributylhexadecylphosphonium bromide).

is observed that the presence of anionic additives (type B), provide more selective units towards less interferents species (sulphate, bicarbonate, nitrate and iodide) while the degree of interference increases for stronger ligands as salicylate and thiocyanate. Between electrode types B and C, with increasing concentrations of NaTPB (10% and 20% mol relatively to the ionophore), the same tendency is observed according to the relative quantity present in membranes. Therefore, the effect of the introduction of anionic species in membranes for potentiometric determination depends strongly on the interfering species present in real samples. The use of EVA as polymeric support instead of PVC also influences the selectivity characteristics of the membranes relatively to other anionic species present in solution (type F). Generally, it is observed (Fig. 5) that the extension of interference is higher for most of the determined interferent anions when compared with those prepared with PVC, except for salicylate and thiocyanate. It was also registered some sequence inversions for species as bicarbonate and nitrate. Fig. 5 also shows that iodide interference becomes positive, while the selectivity of PVC electrodes towards this anion was better.

It is also evident that the developed detectors sensitive to penicillin-G exhibit better characteristics than those previously [5] constructed and based on anion exchangers, namely parameters such as interference level, response-time and sensitivity (Table 4).

3.2. Tubular electrodes behaviour in SIA

Continuous flow methodologies are characterized by the injection of reduced volumes of sample in a flowing stream, which carries the analyte through a chemical detector. In flow injection apparatus, it occurs a physical dispersion of the sample zone within the carrier stream solution, leading to an analytical signal, with reduced intensity, relatively to the one obtained by conventional analysis [25]. Sample zone dispersion depends largely on the aspiration volumes and flow-rates selected, which influences reproducibility and calibration curve parameters. Because of their good analytical characteristics in batch, type A and type B membranes were selected for the construction of tubular electrodes for determination of penicillin-G under flow conditions (types TA and TB), and their behaviour was assessed in the SIA system. To carry out this study, the electrodes were coupled to the sequential injection system depicted in Fig. 2 and evaluated using 0.05 mol l^{-1} NaH_2PO_4 buffer solution as carrier.

Before application of the penicillin-G tubular electrode on the determination of real samples, some flow parameters were optimized. After setting the flow rate at 3.9 ml min^{-1} for 100 s (6.3 ml of propelled solution), injected volumes of sample were investigated within 83 and 278 μl. A volume of 140 μl was selected after considering linearity of the calibration curve, sensitivity and suitable mixing conditions. Volumes smaller than 140 μl provided dispersion of the sample zone and lack of linearity; while higher volumes required more time to return to the baseline and more consumption of reagents. Mixing conditions were evaluated in terms of baseline stability and shape of the analytical signals. In spite of providing a decrease in sampling rate, a propelled volume of 6.3 ml was required for baseline enhancement. Therefore, 100 s were necessary for the complete return of the analytical peak to the baseline. General working characteristics of the electrodes were evaluated by making calibration curves on a range between 5×10^{-5} and 10^{-2} mol l^{-1} (Table 2). It was observed a decrease on the slope on both membrane types, being -59.3 mV dec^{-1} for the membrane without NaTPB

Table 5
Comparison of analytical and system characteristics of penicillin-G selective electrodes in SIA and batch analysis

	SIA[a] type TB	Batch[b] type B
Slope (mV dec^{-1})	-57.3 ± 1.2	-61.1 ± 0.6
LLLR (mol l^{-1})	2.0×10^{-4}	4.9×10^{-5}
PLD (mol l^{-1})	1.5×10^{-4}	3.0×10^{-5}
Reproducibility (mV day^{-1})	±0.2	±0.3
Sampling rate (samples h^{-1})	25	4
Stabilization of electrode response (s)	15	30
Sample volume/analysis (μl)	140	20000
Reagent volume/analysis (μl)	6330	20000

[a] Obtained with type TB membrane.
[b] Obtained with type B membrane.

Table 6
Determination of penicillin-G in commercial products with conventionally shaped electrodes and tubular electrodes

Penicillin-G formulation	HPLC method	Membrane A	Relative error (%)	Membrane B	Relative error (%)	Membrane TB	Relative error (%)
Lentocilin® S2400[a]	794718 ± 27404	808578 ± 52757	1.7	815912 ± 45992	2.7	–	–
Penadur® L-A[a]	889203 ± 35057	860474 ± 32447	−3.2	870170 ± 75726	−2.1	–	–
Prevacilina® forte[a]	535539 ± 21791	490721 ± 17633	−8.4	485567 ± 38193	−9.3	–	–
Prevacilina® mega[a]	899850± 34328	933962 ± 36297	3.8	922453 ± 62101	2.5	895743 ± 34652	−0.4
Atralcilina® Vet10[b]	249.89 ± 7.45	250.73 ± 10.49	0.3	250.09 ± 19.07	0.1	251.90 ± 2.46	0.8
Atralmicina® Vet[b]	152.56 ± 4.62	153.12 ± 5.47	0.4	155.59 ± 4.33	2.0	150.792 ± 2.62	−1.2
Atralmicina® Vet[b]	239.89 ±11.36	240.40 ± 12.98	0.2	238.95 ± 15.77	−0.4	240.12 ± 2.04	0.1

[a] Average (mg g^{-1}) ± standard deviation (n = 4).
[b] Average (mg ml^{-1}) ± standard deviation (n = 4).

(type TA) and -57.3 ± 1.0 mV dec^{-1} for membranes with anionic additive (type TB). When compared to the corresponding conventionally shaped electrodes (types A and B) an increase on the PLD (1×10^{-4} mol l^{-1}) and LLLR (2×10^{-4} mol l^{-1}) values was observed (Table 2).

In comparison to batch procedures, SIA presents several analytical advantages (see Table 5). Sampling rate is six times higher and sample and reagents consumption is smaller than in batch, providing waste production significantly lower.

3.3. Analytical applications

Penicillin-G determinations in different pharmaceutical preparations were performed on the previously prepared samples by direct potentiometry with conventionally shaped electrodes and by SIA with the respective tubular detectors. The conventionally shaped units chosen reverted to type A and B electrodes because they presented increased sensitivity of response (mV dec^{-1}), good potential reproducibility, stability and appropriate pH operational range. In SIA, membrane type TB was the one chosen because it provided results with better reproducibility, as well as lower response-time. In batch determinations all samples were analysed by direct potentiometry method and the averages of the concentrations obtained for each sample and corresponding standard deviation are shown in Table 6. Due to the different solubility of penicillin-G salts and to the fact that tubular electrodes presented a LLLR higher than the corresponding conventionally shaped electrodes (type B), only four samples were determined by sequential injection analysis. Table 6 indicates the results corresponding to the average of four sample measurements obtained by direct potentiometry. The analytical results were compared with those provided by the analysis of the same samples using the HPLC reference method [2]. The results show that there are no significant differences between the methodology proposed with the electrodes constructed and the chromatographic procedure, since the relative errors are inferior to 3.8% (except for Prevacilina® forte, which HPLC results, also differs largely from the labelled values) and reproducibility is of the same order of magnitude.

4. Conclusions

The study undertaken shows that the use of Mn(III)TPP-Cl, as ionophore, dissolved in *o*-NPOE as mediator solvent and immobilized in PVC, can give rise to electrodes with good response characteristics for the penicillin-G, while being of easy construction and simple use. The addition of cationic or anionic additives to the sensory membranes as well as the use of different polymeric supports such as EVA can condition the behaviour of electrodes in respect to slope, operational pH range, reproducibility, stability of response and selectivity. The potentiometric analysis of penicillin-G in simple matrix commercial products using ion selective electrodes is feasible, with a level of precision similar to that attained by HPLC method. Despite SIA being a preferable technique when routine control of formulations is required, some penicillin-G salts solubility limits its applicability to some pharmaceuticals, once tubular electrodes LLLR is higher than those salts solubility. However, the low cost of analysis and the precision of results compared with the results provided by HPLC method justify the use of potentiometry as an alternative analytical technique for the analysis of this type of products.

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