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Potentiometric behaviour of ion selective electrodes based on iron porphyrins: The influence of porphyrin substituents on the response properties and analytical determination of diclofenac in pharmaceutical formulations

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Abstract

The potentiometric response characteristics of diclofenac selective electrodes based on Fe(III) tetraphenylporphyrin-chloride (Fe(III)TPP-Cl) and Fe(III) tetrakis(pentafluorophenyl)porphyrin-chloride (Fe(III)TPFPP-Cl) in different mediator solvents and ionic additives are compared. The sensitivity, working range, detection limit, response mechanism, and selectivity of the membrane sensor show a significant dependence on the type of carrier substituent and on the pH value of the sample solution. Studies performed with different amounts of cationic additive (tetra-*n*-octylammoniumbromide (TOABr)) and anionic additive (sodium tetraphenylborate (NaTPB)) in the membranes allowed the determination of the potentiometric mechanism of action of the used metalloporphyrins. For the analysis of real samples, Fe(III)TPFPP-Cl (type G), prepared in *o*-NPOE, incorporating 10 mol% of TOABr, was used. This potentiometric unit presented a linear response towards diclofenac concentrations between 10^{-5} and 10^{-2} mol l^{-1} ($I = 0.1$ mol l^{-1}) and slopes of about -59 mV dec^{-1}, exhibiting a response time of 10 s in a buffered solution of ammonia–ammonium sulphate with pH 9.9. The potentiometric analysis of sodium diclofenac in pharmaceutical formulations was carried out by direct potentiometry and the obtained results were compared to those provided by HPLC, presenting relative errors inferior to 1.0%.

Keywords: Diclofenac; Ion selective electrodes; Potentiometry; Iron porphyrins

1. Introduction

The proposal of new anion-selective electrodes has been focused on the research of ionophores capable to interact selectively with the anionic species under determination. To date, metalloporphyrins have emerged as one of the most promising classes of compounds to be used as the active material in potentiometric sensors. These organometallic compounds include a porphyrin structure associated to a central metal cation that is directly responsible for the host guest behaviour. Due to the selective coordination of the central metal with the analyte, metalloporphyrins incorporated into plasticized PVC membranes display anion response profiles that significantly differ from those of conventional ion-exchanger sensors [1]. Some authors have demonstrated that changing the central metal in tetraphenylporphyrin from Mn(III) (neutral ionophore) to Sn(IV) (charged ionophore) increases the selectivity toward salicylate [2]. Instead, maintaining the central metal cation (Sn) and changing the porphyrin structure (from tetraphenylporphyrin to octaethylporphyrin) gives also rise to selective membranes that present different properties towards phthalate [3]. Some other studies have been performed aiming the influence of membrane composition (polymeric support, solvent mediator) on the ionophore mechanism of action and on its ability to form dimers [4]. Depending on the charge of the initial metalloporphyrin molecule, it can act as neutral carrier or charged carrier [5],

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being the incorporation of lipophilic cationic or anionic additives essential for the optimization of the membrane composition for such electrodes [6–9]. For charged ionophores, the formation of binuclear complexes (dimerization) is usually observed and results from the ionophore attempt to achieve a neutral charge state, by neutralization of the excess charge of the central metal [10]. Thus, several membranes based on metalloporphyrins have been used on the construction of anion-selective electrodes for the potentiometric determination of various organic species with pharmaceutical interest, namely salicylate [2], ibuprofen [6] 2-hydroxybenzhydroxamate [7], penicillin-G [8] and valproate [9,11].

The introduction of highly electrophilic substituents, in the metalloporphyrin structure, may change the strength of the interaction between the central metal of the carrier complex and the anion to be determined. This could allow a different carrier selective response, being responsible for a different mechanism of action of the ionophore toward the same analyte [12]. In this work, the effect of the introduction of electrophilic substituents in the porphyrin ring, like fluoride, on the potentiometric response characteristics and selectivity of the diclofenac selective membrane electrodes, is evaluated.

The therapeutic action of sodium diclofenac ([*o*-(2,6-dichloroanilino)phenyl]acetate) is based on its ability to serve as a potent inhibitor of cyclooxygenase enzymes (COX1 and COX2), avoiding the production of prostaglandins. As a result, this non-steroidal drug presents anti-inflammatory, analgesic and antipyretic properties [13] being often used as an adjuvant on the treatment of chronic diseases like arthritis and glaucoma. Regarding its therapeutic relevance different methodologies have been proposed for the determination of sodium diclofenac in pharmaceutical formulations, namely spectrophotometry [14–23], fluorimetry [24–27], potentiometry [28–30], chromatography [31–33], and nuclear magnetic resonance spectroscopy [34]. From all those techniques, potentiometric methods, resorting to ion selective electrodes (ISEs) are considered an advantageous analytical tool, especially for the analysis of complex matrices such as pharmaceutical preparations [35], mainly considering its selectivity, price and analytical range. Some potentiometric studies have already been performed using diclofenac selective electrodes based on bathofenantroline [28] and cyclodextrin [30], and some other trials were carried out in non-aqueous media by indirect titration of the mentioned anti-inflammatory agent [29]. However, as far as we know, no diclofenac selective electrode, based on metalloporphyrins, is referred to in literature.

Therefore, tetraphenylporphyrin iron(III) (Fe(III)[TPP]-Cl) and tetrakis(pentafluorphenyl)porphyrin iron(III) (Fe(III)-[TPFPP]-Cl) were incorporated as ionophores in PVC membranes for the determination of diclofenac and cationic and anionic additives were added to some membranes to evaluate their influence in membrane electrodes performance.

The usefulness of the developed potentiometric units was evaluated by their application to the analytical determination of diclofenac in pharmaceutical formulations by direct potentiometry.

2. Experimental

2.1. Reagents and solutions

Analytical grade reagents were used without further purification and all solutions were prepared with distilled and deionized water (conductivity <0.1 $\mu S\,cm^{-1}$).

The ionophores Fe(III)TPP-Cl and Fe(III)TPFPP-Cl were purchased from Aldrich. For the preparation of the selective membranes, *o*-nitrophenyl octylether (*o*-NPOE, Fluka) and dibutylphthalate (DBP, Riedel de-Haen), were used as plasticizers, high molecular weight poly(vinyl chloride) (PVC, Fluka) as immobilising matrix, tetra-*n*-octylammoniumbromide (TOABr, Fluka) and sodium tetraphenylborate (NaTPB, Aldrich) were incorporated as cationic and anionic additives, respectively. The polymeric support was dissolved in tetrahydrofuran (THF, Riedel-de-Haen).

For adjusting simultaneously the pH and ionic strength of the standard solutions and pharmaceutical samples, a buffer solution of ammonia–ammonium sulphate, adjusted to pH 9.9 and $I = 0.1\,mol\,l^{-1}$, was used. A buffer solution of 2-morpholinoethanesulphonic acid/sodium 2-morpholinoethanesulphonate (HMES-MESNa, Fluka), pH 6 (ionic strength $0.1\,mol\,l^{-1}$), was also used to perform some trials.

For the preparation of the sodium diclofenac (Sigma-D-6899) stock solution ($1 \times 10^{-2}\,mol\,l^{-1}$) a rigorous amount of solid was diluted in the previously prepared buffer. The standard solutions for all potentiometric measurements consisted of rigorous dilutions from the former stock solution with the pH and ionic strength adjusting solution.

In the analysis of pharmaceutical samples by the reference HPLC method [36], the mobile phase was a degasified and filtered mixture of methanol (MeOH, Merck) and phosphate buffer (H_3PO_4/NaH_2PO_4), pH 2.5 in a proportion of 70/30 v/v. A 70/30 v/v mixture of methanol and water was used for the dilution of a $0.75\,mg\,ml^{-1}$ diclofenac standard solution in order to obtain $1.5\,\mu g\,ml^{-1}$.

2.2. Sample preparation

Different commercial pharmaceutical formulations containing diclofenac (ampoules, eye drops and tablets) were analysed by direct potentiometry. The liquid samples (ampoules and eye drops) were prepared by dilution with an ammonia–ammonium sulphate buffer solution, guaranteeing pH and ionic strength adjustment. For the solid samples preparation, 10 tablets of each formulation were weighed, disintegrated and an amount of the homogenised mixture corresponding to approximately 15 mg of diclofenac was weighed, suspended in about 50 ml of buffer, set aside for 30 min and filtered. The dilution of each sample was established according to its initial diclofenac content labelled, in order to obtain a final concentration fitted in the linear analytical range of the developed electrodes (about $5 \times 10^{-5}\,mol\,l^{-1}$).

For the reference HPLC method, proposed by USP pharmacopoeia [36], a homogenised aliquot from each sample (ampoules or eye drops) was diluted with the mobile phase to obtain a $0.75\,mg\,ml^{-1}$ sodium diclofenac concentration. The

tablet sample solution was prepared according to the reference procedure [36], by ultrasonic dissolution in a methanol/water (70:30) diluent of a sample amount corresponding to a final diclofenac concentration of 0.75 mg ml^{-1}. These solutions were filtered prior to use. The values obtained for each sample by the HPLC measurements were then compared with those obtained with the standard solution, which had the same content in diclofenac.

2.3. Apparatus and electrodes

A Crison 2002 pH potentiometer (sensitivity ±0.1 mV) coupled to an Orion 605 electrode switcher was used for all the potentiometric measurements.

The indicator electrode was used in conjunction with an Orion double junction Ag/AgCl electrode model 90-02-00, with a solution of the same brand in the internal compartment and a solution of ammonia–ammonium buffer solution (pH 9.9 and $I = 0.1\ mol\ l^{-1}$) in the external compartment. The potentiometric measurements were performed at room temperature (22 °C) in the previously prepared buffer solution. Solutions were magnetically stirred (Crison microST 2038) throughout, being the equilibrium potentials recorded. All the pH measurements were carried out with a Philips GAH 110 glass electrode.

The analysis of the samples by HPLC were carried out in a Merck Hitachi chromatographic system comprising a model 7100 pump, a Rehodyne 7725i injector (20 μl loop) and a Lichrocart RP 18 column (250 mm × 4 mm) packed with Lichrosorb 5 μm beads. A diode array system, model 7455, was used as detector and the data were processed by the software of the same brand, model D7000.

2.4. Membrane preparation and electrode construction

Diclofenac selective electrodes with a PVC membrane and without internal reference solution were constructed as previously described [37]. The sensor solutions were prepared by dissolving the metalloporphyrins (Fe(III)TPP-Cl or Fe(III)TPFPP-Cl) in the plasticizer solvent (*o*-NPOE or DBP). Then, the former solution was added to the PVC dissolved in tetrahydrofuran (THF). PVC-based anion-selective membranes were composed by 1% (w/w) of ionophore, 66% (w/w) of *o*-NPOE or DBP and 33% (w/w) of PVC. To evaluate the response mechanism of the different metalloporphyrins used as ionophores, and the influence on membrane selectivities, different amounts of lipophilic cationic and anionic additives were also incorporated (Table 1).

After the complete drying of the membranes, the electrodes were placed in a $0.01\ mol\ l^{-1}$ solution of diclofenac for 2 h before use, in which they were kept when not in use.

3. Results and discussion

3.1. Evaluation of the electrode response

To evaluate the potentiometric characteristics of ion selective electrodes based on Fe(III)TPP-Cl and Fe(III)TPFPP-Cl metalloporphyrins, two different plasticizers (DBP and *o*-NPOE) and two different additives, with two different amounts, were also considered in the selective membrane preparation (Table 1).

According to the Reilley diagrams obtained for some membranes (Fig. 1), a typical profile was achieved, in which two distinct zones, can be observed, at different pH values. At pH values under 6, extensive precipitation of diclofenac in the form of acid occurs due to the protonation of the secondary amine. Thus, the potential response of all membrane sensors were assessed at pH 6 and 9.9, in diclofenac concentrations in the range of 1×10^{-6} to $1 \times 10^{-2}\ mol\ l^{-1}$, in ammonia–ammonium buffer solution (pH 9.9 and $I = 0.1\ mol\ l^{-1}$) and 2-morpholinoethanesulphonic acid/sodium 2-morpholinoethanesulphonate (pH 6 and $I = 0.1\ mol\ l^{-1}$). Variations concerning to lower limit of linear response (LLLR), practical limit of detection (PLD) and slope are registered, as shown in Table 2 and Fig. 2, when the potentiometric calibration curve is performed at pH 6 and 9.9. Considerable differences, on the response properties of electrodes, prepared only with Fe(III)TPP-Cl or Fe(III)TPFPP-Cl, can be observed, in what concerns to LLLR, PLD and the slope of calibration curve. At pH 9.9 the membrane prepared with Fe(III)TPP-Cl (type A) presented a slope of $-28.1\ mV\ dec^{-1}$ against a slope of $-59.3\ mV\ dec^{-1}$ for the membrane prepared with Fe(III)TPFPP-Cl (type B). Also, type A membrane provided electrodes with a narrower operational range compared

Table 1
Membrane composition (%, w/w) of the constructed electrodes for diclofenac

Electrode type	PVC	Plasticizer		Fe(III)TPP-Cl	Fe(III)TPFPP-Cl	TOABr[a]	NaTPB[a]
		o-NPOE	DBP				
A	33	66		1			
B	33	66			1		
C	33		66	1			
D	33		66		1		
E	33	66		1		10	
F	33	66		1		20	
G	33	66			1	10	
H	33	66			1	20	
I	33	66		1			10
J	33	66			1		10

[a] mol% relative to the ionophore amount.

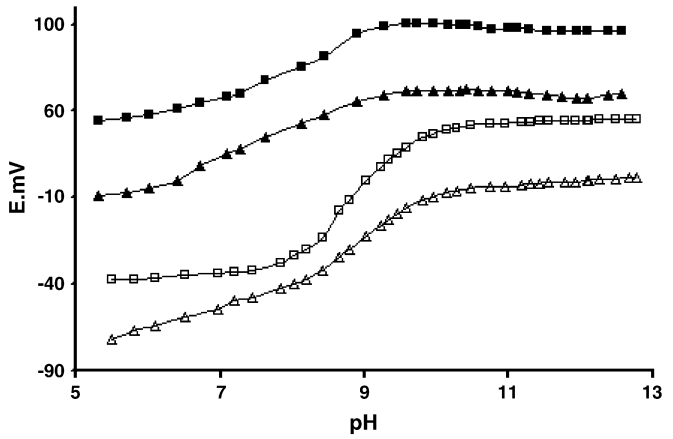

Fig. 1. Electrodes profile vs. pH variation for type E (1×10^{-3} mol l^{-1}) (■) and (1×10^{-2} mol l^{-1}) (□) and for type G (1×10^{-3} mol l^{-1}) (▲) and (1×10^{-2} mol l^{-1}) (△).

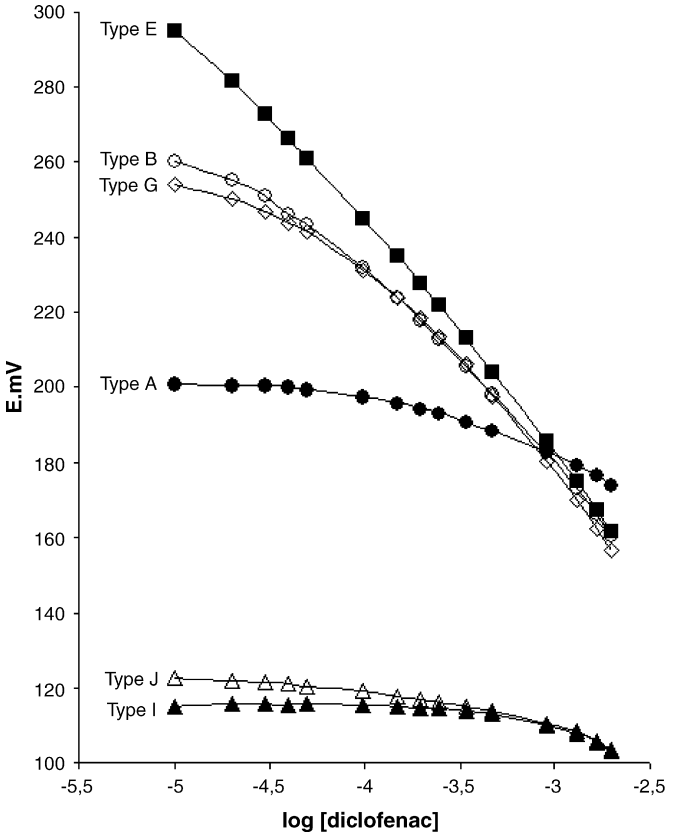

Fig. 2. Typical potentiometric diclofenac responses (in ammonia–ammonium sulphate buffer, adjusted to pH 9.9 and $I = 0.1$ mol l^{-1}) for PVC membranes types A, B, E, G, I and J.

to type B units, since the registered values for LLLR and PLD are about 100 times higher than the values observed for type B membrane. As could be predicted, the presence of fluorine substituents on Fe(III)TPFPP-Cl induce a more positive charge density on the metal centre, thus favouring the extraction and complexation of diclofenac anion into type B sensor membrane. Under more acidic conditions (pH 6) diclofenac anions compete as axial ligands with the hydroxyl groups bridging two molecules of metalloporphyrins (dimers) [38]. With the extraction of the former into membrane, dimer–monomer equilibrium is probably shifted toward monomer porphyrin by simultaneous protonation of the hydroxyl groups [39] and axial bonding of diclofenac molecules. Thus super-Nernstian calibration slopes of −83.1 and −140.0 mV dec^{-1} for type A and type B (more evident) membranes were registered. In order to study the effect of the plasticizer polarity on the electrodes slope, a lower polarity plasticizer (e.g. $\varepsilon_{DBP} = 6.44$ [40] and $\varepsilon_{o\text{-NPOE}} = 24$ [41]) was used to prepare membranes containing Fe(III)TPP-Cl (type C) and Fe(III)TPFPP-Cl (type D). At pH 9.9 types C and D electrodes did not present a potentiometric response, probably because in a less polar environment dimer formation does not exist and OH^- anions may act as primary ion. At pH 6 type D electrode present a worse response than type C, maybe because dimer–monomer equilibrium plays a more important role in the potentiometric response of the porphyrin with a more positive metallic centre (Fe(III)TPFPP-Cl). As the presence of DBP originated potentiometric units with poorer characteristics (slope, LLLR and PLD) relatively to those prepared with *o*-NPOE, only this last plasticizer was used for the construction of polymeric membrane electrodes with lipophilic additives.

According to literature, metalloporphyrins with a +3 oxidation state in the central metal, can act either by a neutral or charged response mechanism, depending on the specific nature of the axial ligands. In the case of a neutral carrier mechanism, the addition to the membrane of ionic sites with an opposing charge to that presented by the primary ion, is necessary to improve analyte extraction into the membrane thus

Table 2
General working characteristics of the constructed diclofenac ISEs

Electrode type	Slope (mV dec^{-1}) pH 6/pH 9.9	LLLR (mol l^{-1}) pH 6/pH 9.9	PLD (mol l^{-1}) pH 6/pH 9.9
A	−83.1 ± 0.8/−28.1 ± 1.1	2.0×10^{-4}/1.0×10^{-3}	1.5×10^{-4}/9.9×10^{-4}
B	−140.0 ± 0.8/−59.3 ± 0.4	2.0×10^{-5}/2.0×10^{-5}	1.0×10^{-5}/1.5×10^{-5}
C	−68.8 ± 0.9/–	2.5×10^{-4}/–	2.0×10^{-4}/–
D	−37.3 ± 1.2/–	3.5×10^{-4}/–	2.5×10^{-4}/–
E	−83.2 ± 0.9/−59.2 ± 0.3	9.9×10^{-6}/9.9×10^{-6}	8.0×10^{-6}/8.0×10^{-6}
F	−85.5 ± 0.8/−62.5 ± 0.3	9.9×10^{-6}/9.9×10^{-6}	8.0×10^{-6}/8.5×10^{-6}
G	−79.2 ± 1.2/−59.8 ± 0.3	1.0×10^{-4}/2.0×10^{-5}	9.9×10^{-5}/1.5×10^{-5}
H	−78.5 ± 1.3/−61.0 ± 0.4	1.0×10^{-4}/2.0×10^{-5}	9.9×10^{-5}/1.5×10^{-5}
I	−44.6 ± 1.2/−16.7 ± 0.2	1.0×10^{-3}/9.0×10^{-4}	9.9×10^{-4}/5.0×10^{-4}
J	−120.2 ± 0.6/−11.9 ± 0.3	1.0×10^{-5}/4.5×10^{-4}	8.0×10^{-6}/4.0×10^{-4}

rendering electrodes with improved sensitivity [2]. On the other hand, for a response based on a charged mechanism, addition of sites with the same charge of the primary ion, is required to improve the selectivity of the sensor. Hence, the introduction of lipophilic ionic sites in membranes was carried out to determine the real operative mechanism of the constructed electrodes and also to develop units with better potentiometric characteristics. Some membranes were prepared by the introduction of different amounts of cationic (types E, F, G and H) and anionic sites (types I and J). Regarding the electrodes prepared with Fe(III)TPP-Cl at pH 9.9, considerable changes were observed by the addition of ionic species. The incorporation of NaTPB lipophilic anionic additive (type I) to membranes rendered unstable electrodes that did not show a potentiometric response towards diclofenac. On the other hand, the addition of TOABr (lipophilic cationic species) to membranes (types E and F) clearly improved the potentiometric characteristics of the developed electrodes, relatively to those units without ionic sites. In fact, the slope increased from about $-28\,mV\,dec^{-1}$ for type A to a Nernstian slope of $-59.2\,mV\,dec^{-1}$ for type E membrane, with 10 mol% of TOABr. The operative range of the electrodes was also favoured by the addition of lipophilic cationic additives to membranes (see Table 2). However, increasing the amount of TOABr from 10 mol% (type E) to 20 mol% (type F) did not considerably change the potentiometric behaviour of the units. Table 2 also shows the results obtained with Fe(III)TPP-Cl, at pH 6, for the same units (types E, F, and I): slope values did not suffer considerable changes by the addition of TOABr but PLD and LLLR presented lower values. Like at pH 9.9, from the addition of lipophilic anionic additive (NaTPB) to membranes (type I), electrodes sensitivity was visibly diminished, being registered a sub-Nernstian slope of $-44.6\,mV\,dec^{-1}$ and higher PLD and LLLR, suggesting a neutral operational mechanism of action for Fe(III)TPP-Cl. The same studies were performed with electrodes prepared with Fe(III)TPFPP-Cl (types B, G, H and J) using two pH conditions and different results were revealed. The introduction of NaTPB, as anionic species, into membranes (type J) also gives rise to electrodes which do not exhibit a potentiometric response towards diclofenac, at pH 9.9, but with similar characteristics relatively to those units without additives at pH 6. However, the introduction of lipophilic cationic additive (TOABr) to membranes (types G and H) did not so clearly improve the electrodes performance, such as it happened for electrodes doped with Fe(III)TPP-Cl (type E). At pH 6, the introduction of TOABr provided electrodes with considerably lower slopes and higher LLLR and PLD values when compared to type B membrane in the same pH conditions. This behaviour could be due to the presence of the electrophilic fluorine substituents in the porphyrin ring of Fe(III)TPFPP-Cl, which leads to increased acidity of the ionophore. At pH 6, this ionophore is capable of acting as a charged ionophore, being water molecules the most important axial ligand present in solution [39]. However, at pH 9.9, both axial ligands are anionic (hydroxide and chloride) and the ionophore may act as neutral carrier. Hence, it was possible to conclude that both metalloporphyrins act as neutral at pH 9.9, but Fe(III)TPFPP-Cl may act also as a charged carrier at pH 6. Taking into account the results obtained at pH 6 and 9.9, especially regarding slope values and for comparison purposes considering a unique type of working mechanism for the two studied metalloporphyrins, selectivity and analytical applications were further exploited at pH 9.9.

3.2. pH influence

It was previously referred that electrodes based on Fe(III)TPP-Cl and Fe(III)TPFPP-Cl may present different responses according to the various pH conditions selected for the measurements, especially slope values. Aiming the study of the pH influence on types E and G electrodes, their potential changes versus the pH were recorded in diclofenac solutions of 10^{-3} and $10^{-2}\,mol\,l^{-1}$. The pH changes were obtained by the addition of small volumes of sodium hydroxide and concentrated sulphuric acid solutions and recorded with a glass electrode. For low pH values diclofenac started to precipitate in the form of acid, by protonation of the secondary amine and for that reason the study was performed in a pH range of 6–13. The Reilley diagrams (Fig. 1) of the evaluated electrodes show that the potential values did not vary by more than ±5 mV within the pH range 8.8–12.8 for both potentiometric units. Metalloporphyrins are known as ionophores that present a significant dependence on the pH of the solution, especially for higher values where the concentration of OH^- increases and it becomes interfering species [42]. However, in the present study, the primary ion, diclofenac, seems to have an especial ability to coordinate to the neutral ionophore, since no influence is observed by the increase of OH^- in solution (Fig. 1).

3.3. Electrode selectivity

Selectivity is one of the most important characteristics of ISE that is expressed by the potentiometric selectivity coefficients ($\log K^{pot}$) and describes the preference by the electrode membrane for an interferering ion relative to the primary ion (diclofenac). The interference extent of several inorganic and organic anions, in membrane electrodes based on Fe(III)TPP-Cl and Fe(III)TPFPP-Cl, was evaluated by determining the potentiometric selectivity coefficients by the separated solutions method [43] with interfering and primary ion at various concentration levels, all under the same experimental conditions (in background ammonia–ammonium sulphate, pH 9.9, ionic strength $0.1\,mol\,l^{-1}$). Results regarding $5 \times 10^{-3}\,mol\,l^{-1}$ solutions are plotted in the diagram represented in Fig. 3, for electrodes types B, E, F, G and H. Type A membrane, without additive, was not tested because presented poor potentiometric characteristics at pH 9.9. None of the investigated species were found to interfere significantly on the constructed electrodes, reflecting a very high selectivity of those units towards diclofenac. The increase of TOABr from 10 to 20 mol% in membranes prepared with Fe(III)TPP-Cl (types E and F) did not considerably change the selectivity sequence of the units, which presented a selectivity pattern very similar to the Hofmeister one ($SCN^- > Sal^- > NO_3^- > NO_2^- > Cl^- > CH_3COO^-$) (typical of the classical ion-exchange sensors), except in the sequence between chloride and acetate. Relatively to

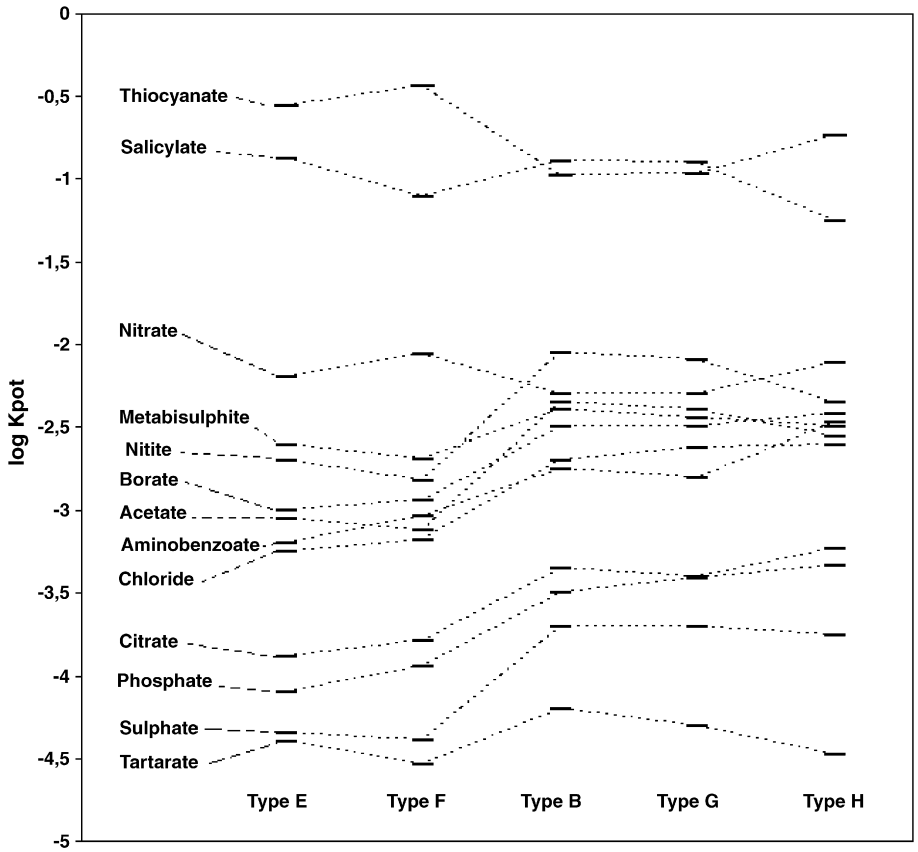

Fig. 3. Selectivity profiles of types E, F, B, G and H units in ammonia–ammonium sulphate, adjusted to pH 9.9 and $I = 0.1\ \mathrm{mol\,l^{-1}}$.

membranes based on Fe(III)TPFPP-Cl (types B, G and H) some significant changes are registered. It can be observed that the sequence of selectivity for type B unit ($Sal^- > SCN^- > NO_2^- > NO_3^- > CH_3COO^- > Cl^-$) is quite different from the so-called Hofmeister pattern. For metalloporphyrins acting as ionophores, besides the electrostatic interaction between the central metal and anions, there is a selective coordination capability, favouring the interaction of less lipophilic anions [44]. Thus, the selectivity profile can be quite different from the classical ion-exchange type electrodes, like quaternary ammonium salts, in which the more lipophilic species are the preferred ones. The incorporation of 10 mol% of TOABr into membranes (type G) did not change the selectivity sequence, indicating a prevalence of the coordination mechanism, but increasing that amount to 20 mol% (type H) thiocyanate becomes the main interfering species and nitrate becomes more interferent than nitrite, suggesting a dominance of the ion-exchanger mechanism. Comparing types E and G electrodes, with the same amount of TOABr, but different metalloporphyrins, it is possible to conclude that Fe(III)TPP-Cl provides electrodes with selectivity sequences more similar to the Hofmeister pattern, which means that the ion-exchange mechanism, due the presence of TOABr, probably overlaps the coordination mechanism with the central metal of Fe(III)TPP-Cl. Maybe the absence of withdrawing groups like fluorine atoms, favours the ion-exchange mechanism by avoiding the increase of the positive charge of the central metal, which could somewhat help the coordination mechanism with the analyte.

Taking into account the results obtained in this section, further studies will be performed with G units.

Table 3
Determination of diclofenac in pharmaceutical formulations (mg diclofenac/unit)

Sample	Pharmaceutical formulation	HPLC method[a]	Type G membrane[a]	Relative error (%)
1	Flameril® 75 mg/3 ml	74.9 ± 0.5	74.9 ± 0.5	0.0
2	Olfen® 75 mg/3 ml	75.3 ± 0.5	75.1 ± 0.6	−0.30
3	Fenil V® 75 mg/2 ml	75.2 ± 0.4	75.0 ± 0.5	−0.30
4	Voltaren® 75 mg/2 ml	74.9 ± 0.3	74.9 ± 0.4	0.0
5	Voltaren® 1.0 mg/ml	0.99 ± 0.01	0.99 ± 0.01	0.0
6	Diclofenac merck® 50	50.0 ± 0.4	50.2 ± 0.3	0.40

[a] Average ± S.D. ($n = 4$).

Table 4
Validation of the proposed method by linear regression analysis and calculation of the Student's *t*-test

	Linear regression analysis	Paired two-tail Student's *t*-test at 95% confidence level
Type G	$y = 0.99\ (\pm 0.002) + 0.07\ (\pm 0.15)$	0.61

3.4. Analytical applications

For the determination of diclofenac in liquid pharmaceutical preparations (injectables and eye drops) type G electrodes were selected as they presented good selectivity and sensitivity and had a similar formulation using both types of the studied metalloporphyrins. A solid sample (tablets) was also evaluated to assess the utility of the developed electrodes in a different matrix. The drug assay was carried out by direct potentiometry, following the preparation previously described, using a standard curve constructed with pure diclofenac solutions.

Table 3 indicates the average of four measurements obtained by direct potentiometry for the selected potentiometric unit. The results obtained were compared with those provided by the analysis of the same samples using the HPLC reference method [33] (Table 4) and are in good agreement with those obtained by the chromatographic procedure, since relative errors are inferior to 1%.

4. Conclusions

The presence of electrophilic substituents plays an important role on those metalloporphyrins operative mechanism, which is also strongly dependent on the pH of the solution. For the determination of the mechanism of action of those ionophores it was necessary the addition of cationic or anionic lipophilic sites to the sensory membranes. At pH 9.9 both membranes exhibited a neutral mechanism of action, being necessary the introduction of lipophilic cationic sites. However, at pH 6, membranes doped with Fe(III)TPFPP-Cl could act as charged carriers due to the electron withdrawing capacity of fluorine atoms as substituents, being necessary the introduction of NaTPB to membranes, to display a better potentiometric performance. The existence of super-Nernstian slopes was attributed to dimers formation as a consequence of the different plasticizers used and also to the pH of the solution.

The present study shows that the use of iron porphyrins on the development of diclofenac selective electrodes may constitute an alternative and advantageous technique for this drug determination in pharmaceutical samples. The potentiometric analysis of diclofenac in pharmaceutical formulations is feasible, with a level of precision similar to that obtained with a HPLC reference method, justifying the proposed methodology as an alternative analytical technique for the analysis of this drug.

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