

Chloride-selective membrane electrodes and optodes based on an indium(III) porphyrin for the determination of chloride in a sequential injection analysis system

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Received 23 January 2004; received in revised form 23 January 2004; accepted 25 April 2004

Available online 2 July 2004

Abstract

Two quasi-independent methods for potentiometric and optical determination of chloride were simultaneously implemented in a flow system, providing real-time assessment of the quality of results. A potentiometric and an optical polymeric membrane doped with the same indium(III) octaethyl-porphyrin were used as sensor ionophore. The working mechanism and the analytical characteristics of these porphyrin-based sensors with respect to dynamic range, selectivity, repeatability and lifetime are discussed. These sensors, utilised as detectors in a flow system, were applied for the analysis of chloride in pharmaceutical solutions. The quality of the results obtained was evaluated by comparison with those provided by the reference method and no significant statistical differences at the 95% confidence level were observed. The simultaneous attainment of two measurements permitted the standardisation of results in real time and the detection of failures in the procedure.

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Keywords: Sequential injection analysis; Real-time accuracy assessment; Chloride-selective electrode; Chloride optode; Indium(III) octaethyl-porphyrin

1. Introduction

The accuracy assessment concept in flow analysis has been proposed and applied in the determination of several analytes in large scale analysis [1–4]. In these systems, the injected sample is processed by two quasi-independent analytical methods, thus enabling intrinsically more accurate results. As two measurements of the same analyte of the injected sample are obtained at the same time their comparison can be exploited for accuracy assessment. If the two measurements are similar the quality of the results is considered good. If the two measurements disagree, failure of the system performance and/or a matrix interference can be inferred. Taking into account that random errors should not affect both measurements with the same sign and magnitude and that the systematic deviations are different in both methods, the value corresponding to their mean is a

more accurate estimate of the analyte concentration in the sample. Other figures of merit of flow systems implementing this concept are the low consumption of reagents and high sampling rate, thus rendering good analytical tools for process control [5]. In this paper, the application of the concept of real time accuracy assessment in a SIA system is proposed by using two chemical sensors based on the same recognition element, employing, however, two different transduction procedures (potentiometric and optical). The recognition membranes were composed of an indium(III) porphyrin immobilised on poly(vinyl chloride) (PVC) film, and their analytical usefulness were evaluated in the determination of chloride in pharmaceutical formulations. Indium(III) porphyrins have been used as anion carriers for ion-selective electrodes [6–9] and optodes [10–14] based on polymeric membranes, giving enhanced responses toward chloride ion. Moreover, they exhibit selectivity profiles which deviate significantly from the Hofmeister sequence, due to the preferred axial site coordination of anions with the metallic centre of the metalloporphyrine structure.

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2. Experimental

2.1. Reagents and solutions

All solutions were prepared with deionised water (conductivity $<0.1 \mu\text{S cm}^{-1}$) and analytical grade reagents were used without further purification.

The ionophore chloro (2,3,7,8,12,13,17,18-octaethylporphyrinato) indium(III) (In(OEP)Cl) was synthesised via metallation of the corresponding free porphyrin (Aldrich 25,240-9) with the metal(III) salt (Aldrich 33,406-5), which was carried out by reflux in glacial acetic acid, according to the procedure described previously [15,16]. Metalloporphyrin purity was confirmed by thin layer chromatography. Membrane components poly(vinyl chloride) (PVC), 4',5'-dibromofluorescein octadecyl ester (ETH 7075), potassium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (KTFPB), *o*-nitrophenyloctyl ether (*o*-NPOE), and bis(2-ethylhexyl)sebacate (DOS) were used as received from Fluka. The solid polymers were dissolved in tetrahydrofuran (THF, Merck-1.09731) for preparation of the membranes.

Simultaneous adjustment of pH and ionic strength was accomplished by using an $0.05 \text{ mol l}^{-1} \text{ NaH}_2\text{PO}_4$ buffer solution (pH 4.2) as carrier solution in the flow system.

The chloride reference solutions were prepared by appropriate dilution, with the buffer solution, of a $0.1000 \text{ mol l}^{-1} \text{ NaCl}$ stock solution. The silver nitrate titrant solution used in the reference procedure [17] was standardised against a chloride standard solution by potentiometric titration.

The determination of chloride content in pharmaceutical formulations by the proposed and reference procedures was performed after appropriate sample dilution in order to obtain final concentrations of ca 1.0×10^{-3} , 2.5×10^{-2} , $1.0 \times 10^{-2} \text{ mol l}^{-1}$ of chloride ion. Samples were diluted with a $0.05 \text{ mol l}^{-1} \text{ NaH}_2\text{PO}_4$ buffer solution.

2.2. Construction of the ion-selective electrodes

The polymeric membranes used for the construction of potentiometric detectors were prepared by dissolving In(OEP)Cl (1.0%, w/w) and KTFPB (0.4%, w/w = 32% mol, relative to metalloporphyrin) in the mediator solvent, *o*-NPOE (65.6%, w/w). Thereafter, the PVC (33.0%, w/w) was dissolved in THF and added to the previous mixture. Chloride-selective electrodes, without an inner reference solution, were constructed in a tubular configuration as previously described [18]. The membrane solution was directly applied on the conductor support, made up of a mixture of epoxy resin (Araldite[®]) with graphite powder [19], and the THF solvent was left to evaporate completely in air. Finally, the electrode was placed in a $1.0 \times 10^{-4} \text{ mol l}^{-1}$ sodium chloride solution for membrane hydration.

2.3. Construction of the ion selective optode

The optical chloride membrane was composed of 30%, w/w PVC, 1.8%, w/w of In(OEP)Cl, 1.0%, w/w of ETH 7075 and 67.2%, w/w of DOS. Blank reference membranes (without ionophore and chromoionophore) with similar composition (30%, w/w of polymer and 70%, w/w of DOS) were also prepared. The film casting solutions were prepared by dissolving a total of 200 mg of membrane components in 2 ml of THF. Optical sensing membranes were cast from these solutions on to glass slides (9 mm \times 40 mm, 1 mm thickness for batch measurements, and 18 mm \times 18 mm, 1 mm thickness for flow measurements) by spin coating at 2000 rpm. The THF was allowed to evaporate overnight, leaving a thin polymer film attached to the glass slide.

2.4. Apparatus

A micropH decimillivoltmeter (Crison model 2002, sensitivity $\pm 0.1 \text{ mV}$) was used to measure the potential differences between a double-junction AgCl/Ag reference electrode (Russel model 90-0029) and the indicator electrode. The outer compartment of the reference electrode was filled with a $0.05 \text{ mol l}^{-1} \text{ NaH}_2\text{PO}_4$ solution.

Batch UV–Vis absorbance measurements were made on a UV–Vis double beam spectrophotometer (Lambda 6B, Perkin-Elmer), with the polymer film coated glass slides placed in a commercial cubic quartz cell (1 cm \times 1 cm \times 4 cm) containing the test solution. The films were soaked for 20 min in $0.05 \text{ mol l}^{-1} \text{ NaH}_2\text{PO}_4$ solution (pH 4.2) before the measurements. Flow absorbance measurements were made on a UV–Vis spectrophotometer (Jenway) using a flow-through cell with a volume of 150 μl and a path-length of 3 mm. Two identical membranes attached on their respective glass slides were mounted in the flow-through cell and placed in the sample beam. Absorbances were measured at 530 nm.

Sequential injection analysis determinations were carried out with the multi-task flow-system (Fig. 1), whose mode of functioning has been described previously [20]. This system was composed of a Gilson Minipuls 3 peristaltic pump (Villier-le-Bell, France), equipped with a PVC propulsion

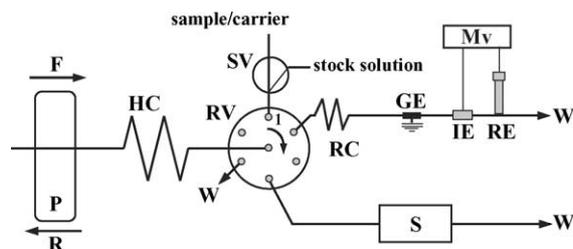


Fig. 1. SIA system for chloride determination in pharmaceuticals. P: peristaltic pump; RV: rotary valve; SV: three-way solenoid valve; HC: holding coil; RC: reaction coil; GE: ground electrode; IE: chloride-selective membrane electrode; RE: reference electrode; Mv: decimillivoltmeter; S: spectrophotometer; W: waste.

tube (Gilson, 1.30 mm internal diameter); a six-port rotary valve (RV) from Valco Instruments, model Cheminert C15-3186E (Houston, USA); and a NResearch 161 T031 (Stow, USA) three-way solenoid valve (SV). The components of the manifold were interconnected with PTFE tubing of 0.8 mm internal diameter. Home-made devices, such as joint pieces, grounding electrode, and supports for tubular and reference electrodes were also employed and constructed as described elsewhere [21]. The system was controlled by a microcomputer through an Advantech PCL 711B interface card. Software for control of the system and data acquisition was written in Microsoft QuickBasic 4.5.

The reference procedure [17] for the determination of chloride consisted of a potentiometric titration with a silver nitrate titrant solution, using as a detector a silver electrode with a homogeneous crystalline membrane, constructed according to Ferreira et al. [22].

2.5. Procedures

In the multi-task system (Fig. 1), the holding coil (HC) and the transmission channels between RV and the detec-

tion systems were initially filled with carrier/ionic strength adjuster solution by selecting the ports 2/4 (potentiometric/optical detection systems, respectively) and setting the peristaltic pump in the propulsion mode, which was maintained until stable baselines were obtained for the two detectors. The instructions required for an analytical determination cycle of chloride are defined in Table 1. The first three steps of the calibration procedures and the first two steps of the determination procedures for both detection systems correspond to the change of the solutions that access the flow system through the SV valve. The selection of port 1 of RV (first step of calibration cycle), while the SV valve was turned on, enabled the filling up of the active access channel with a $2.0 \times 10^{-3} \text{ mol l}^{-1}$ sodium chloride stock solution for the electrode calibration procedure or with a $5.0 \times 10^{-2} \text{ mol l}^{-1}$ sodium chloride stock solution for the optode calibration procedure. When the SV valve was off, the passive port channel was filled either with carrier solution (for the calibration procedure) or with sample solution (in the case of analytical determinations).

The electrode and optode calibration procedures were carried out by preparing in-line reference solutions of dif-

Table 1

Steps for chloride determination in pharmaceutical formulations with the SIA system depicted in Fig. 1

RV	SV state ^a	Volume (μl)	Flow rate (ml/min)	Flow direction	Event
Chloride selective electrode calibration					
1	On	200	1.5	R	Stock chloride solution
1	Off	200	1.5	R	Carrier solution
5	–	600	4.0	F	Waste
1	On	375	2.4	R	Inline chloride reference solution preparation
	25%				
	50%				
	75%				
	100%				
2	–	6000	6.0	F	Chloride measurement
Chloride potentiometric determination					
1	Off	200	1.5	R	New sample
5	–	400	4.0	F	Waste
1	Off	375	2.4	R	Chloride sample volume
2	–	6000	6.0	F	Chloride measurement
Chloride optode calibration					
1	On	200	1.5	R	Stock chloride solution
1	Off	200	1.5	R	Carrier solution
5	–	600	4.0	F	Waste
1	On	650	1.3	R	Inline chloride reference solution preparation
	25%				
	50%				
	75%				
	100%				
4	–	700	1.4	F	Chloride solution in the flow cell
4	–	–	–	–	Flow stopped for 50 s
4	–	6750	2.7	F	Chloride measurement
Chloride optical determination					
1	Off	200	1.5	R	New sample
5	–	400	4.0	F	Waste
1	Off	650	2.3	R	Chloride sample volume
4	–	700	1.4	F	Chloride solution in the flow cell
4	–	–	–	F	Flow stopped for 50 s
4	–	6750	2.7	F	Chloride measurement

RV: rotary valve; SV: solenoid valve; R: reverse; F: forward.

^a Each SV cycle of 2 s.

ferent concentrations [20], as described in the last two steps of the calibration procedures shown in Table 1. Each reference solution was prepared by aspirating into the holding coil, through port 1 of RV, appropriate aliquots of carrier and stock chloride solutions, by means of on/off cycles of SV valve. In the calibration of the potentiometric detector, a $2.00 \times 10^{-3} \text{ mol l}^{-1}$ chloride stock solution was employed, as pointed out above. Five on/off cycles of SV valve, in which SV valve was turned on for 25, 50, 75 and 100% in each cycle, permitted that a total of 375 μl of carrier/chloride stock solutions were aspirated into the holding coil, producing 5.00×10^{-4} , 1.00×10^{-3} , 1.50×10^{-3} , $2.00 \times 10^{-3} \text{ mol l}^{-1}$ chloride reference solutions, respectively. Thereafter, the mixture zone was pumped towards the detector through port 2 of RV at a flow rate of 6.0 ml min^{-1} . For optode calibration, 15 on/off cycles of SV valve were performed, aspirating into the holding coil a total volume of 650 μl , which was subsequently pumped towards the detector through port 4 of RV at a flow rate of 2.7 ml min^{-1} . When the mixture zone reached the optode, the flow was stopped for 50 s and, after signal acquisition, the solution was discharged. In the optode calibration, by using a $5.00 \times 10^{-2} \text{ mol l}^{-1}$ chloride stock solution, 1.25×10^{-2} , 2.50×10^{-2} , 3.75×10^{-2} , $5.00 \times 10^{-2} \text{ mol l}^{-1}$ reference solutions were prepared, by turning on the SV valve for 25, 50, 75 and 100% of the time of each cycle, respectively.

In the determination step, 375 μl and 650 μl of sample solution were aspirated into the holding coil for potentiometric and optical detection, respectively.

3. Results and discussion

3.1. Optimisation of the sensing membranes

In order to obtain sensing membranes with the best characteristics under flow conditions, different formulations were tested by varying the mediator solvent and the ionic additive. For potentiometric purposes, a membrane composed of 1.0%, w/w In(OEP)Cl, 0.4%, w/w KTFPB, 65.6%, w/w *o*-NPOE and 33%, w/w PVC was selected. As previously shown [9], the incorporation in the membrane composition of an anionic lipophilic additive in the proportion of 32% mol/mol relative to metalloporphyrin, resulted in a significant improvement of slope and selectivity of the electrodes when compared with those in which the additive is absent.

For optical membrane preparation, the more lipophilic mediator solvent (DOS) revealed better transparency and a lower leaching profile than *o*-NPOE. For the same reasons the ETH 7075 chromoionophore was selected instead of other ones previously mentioned [10]. A film composed of 1.8%, w/w In(OEP)Cl, 1.0%, w/w ETH 7075, 67.2%, w/w DOS and 30%, w/w PVC was finally selected.

Table 2

Anion selectivity coefficients for the ion selective membrane electrode and for the optical sensing membrane, measured in phosphate buffer solution at pH 4.2

Anions	Log $K_{\text{Cl},\text{Y}}^{\text{Pot}}$ (In(OEP)Cl ISE) ^a	Log $K_{\text{Cl},\text{Y}}^{\text{Opt}}$ (In(OEP)Cl/ETH7075 optode) ^b
Cl ⁻	0	0
Sal ⁻	0.96	0.94
SCN ⁻	0.88	0.62
I ⁻	0.60	0.78
Br ⁻	0.08	0.17
NO ₂ ⁻	0.45	-0.67
OAc ⁻	-0.94	- ^c
ClO ₄ ⁻	-0.87	- ^c
NO ₃ ⁻	-1.3	- ^c
F ⁻	-1.9	- ^c

^a Log $K_{\text{Cl},\text{Y}}^{\text{Pot}}$ calculated at $5.00 \times 10^{-4} \text{ mol l}^{-1}$ Cl⁻ and interferent ion solution.

^b Log $K_{\text{Cl},\text{Y}}^{\text{Opt}}$ calculated at $\alpha = 0.5$.

^c No interference was observed.

3.2. Potentiometric determination

The performances of the ion selective membranes developed were assessed by using the sequential injection system depicted in Fig. 1, in which a 0.05 mol l^{-1} NaH₂PO₄ solution (pH 4.2 and $I = 0.05 \text{ mol l}^{-1}$) was used as carrier solution. A reference/sample solution volume of 375 μl and a flow rate of 6.0 ml min^{-1} were selected, as these conditions enable the attainment of an analytic signal as intense as those obtained by sampling larger volumes, which correspond to the stationary state. The membrane response for different anions was evaluated and the potentiometric selectivity coefficients were determined by the separate solutions method [23], fixing the concentrations of chloride and interfering ions at $5.0 \times 10^{-3} \text{ mol l}^{-1}$. The results listed in Table 2 demonstrate that the electrodes developed follow the typical behaviour of metalloporphyrin-based sensors, presenting a selectivity which deviates from the classical Hofmeister sequence, as they are more selective towards chloride than to more lipophilic ions, such as perchlorate and nitrate. Moreover, when compared with other electrodes based on ion exchangers [24], they reveal better selectivity characteristics for salicylate, thiocyanate and iodide ions. Considering the use of In(III) porphyrins in polymeric membranes, it was also shown that the incorporation of a lipophilic anionic additive induces an anionic charged response mechanism, enhancing the performance and selectivity towards chloride ions [7]. The electrodes also exhibit super-Nernstian calibration curves for chloride ions, within the concentration range from 1.00×10^{-5} to $1.00 \times 10^{-2} \text{ mol l}^{-1}$, with a slope of $-87.5 \pm 1.1 \text{ mV per decade}$, as shown in Fig. 2. This super-Nernstian behaviour is explained by the dimer-monomer equilibrium of the porphyrin into the organic phase of the sensor membrane [9,25]. In the absence of the analyte, the porphyrin is positively charged in the membrane, forming with hydroxide ion a bridged dimeric porphyrin (In(III) [OEP]₂ (OH)⁺),

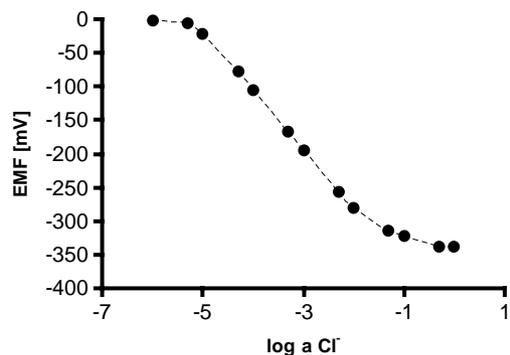
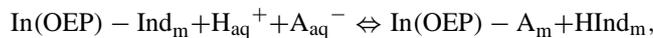


Fig. 2. Potentiometric response to chloride of the selective membrane electrode prepared with a membrane containing 1%, w/w of In(OEP)Cl and 0.4%, w/w of KTFPB in PVC/o-NPOE (33.0; 65.6%, w/w) in NaH₂PO₄ buffer solution, pH 4.2.

with the borate ion acting as the counter ion. However, in the presence of anions which compete for axial bonding with metal centre, the dimeric form is broken yielding porphyrin monomers. The repeatability of the potentiometric detection system was evaluated by processing ten replicates of chloride reference solutions with concentrations of $5.00 \times 10^{-5} \text{ mol l}^{-1}$ and $1.00 \times 10^{-2} \text{ mol l}^{-1}$, which provided relative standard deviations of 0.64 and 0.29%, respectively. The flow system allowed a frequency of 60 samples per hour and the response of the ion selective membrane remained constant for at least four months.

3.3. Optical determination

Indium(III) porphyrins, which were firstly used as sensors for potentiometric detection [6–9], have been also exploited in the development of optical sensors [10–14]. In the present work, a PVC-based membrane, doped with In(OEP)Cl (ionophore) and with a dibromofluoresceine lipophilic derivative (pH indicator) was assessed. When the membrane is immersed in a chloride solution (prepared in phosphate buffer, pH 4.2), the simultaneous co-extraction of the anion and proton occurs in order to assure the electroneutrality of the membrane. As a result, the protonation of the pH indicator is observed, leading to a change in the absorbance spectrum of the membrane. The equilibrium mechanism can be described as:



where Ind_m represents the anionic form of the indicator linked to In(OEP) in the absence of the analyte, and HInd_m represents the protonated form of the indicator after the co-extraction into the membrane of the proton H_{aq}⁺ and the anion A_{aq}⁻.

Fig. 3 depicts the absorbance spectra of the sensing membrane in the presence and absence of chloride solution prepared in phosphate buffer (pH 4.2). In the absence of the analyte, the spectrum presents an intense shoulder around 530 nm that is probably due to the bonding established be-

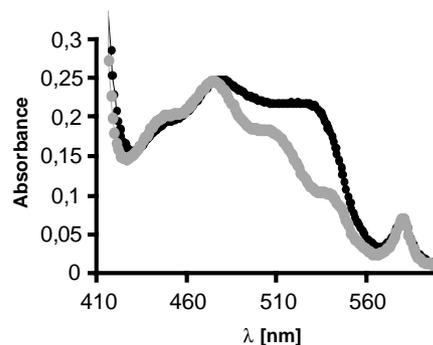


Fig. 3. Absorption spectra of an In(OEP) doped PVC film immersed in a pH 4.2 NaH₂PO₄ buffer solution (a) without and (b) with $1.00 \times 10^{-1} \text{ mol l}^{-1}$ chloride. Membrane composition: 1.0%, w/w ETH 7075, 1.8%, w/w In(OEP)Cl, 67.2%, w/w of DOS, and 30%, w/w PVC.

tween the phenol group of the pH indicator and the porphyrin central atom. However, in the presence of chloride, or other anions that also compete for the axial bonding site, the indicator is released to the bulk of the sensing membrane in the protonated form, decreasing, as a consequence, the absorbance in the region around 530 nm.

After selection of the optimum wavelength for optical transduction, the performances of the optodes developed were evaluated employing the sequential injection flow system. The flow-through cell was assembled by placing two sensing membranes between two black acrylic supporting blocks, separated by a silicone spacer containing two drilled tubular entrances. The flow cell was then placed in the sample compartment of the spectrophotometer and connected to port 4 of RV. As described in the experimental section, 650 μl of the analyte solution were aspirated into the holding coil, propelled towards the detector and the flow was stopped for 50 s when the sample zone reached the flow cell. By this way, an absorbance signal with an intensity of 95% of the corresponding signal obtained in batch conditions was attained. As can be seen in Fig. 4, a dynamic range from 1.00×10^{-3} to 1.00 mol l^{-1} for chloride ions was obtained, by performing a calibration using reference chloride solutions prepared in phosphate buffer (pH 4.2).

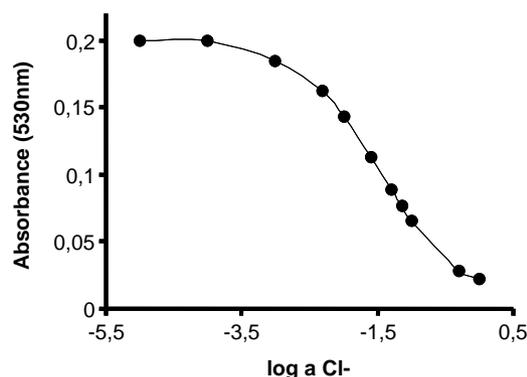


Fig. 4. Optical response of ETH 7075/In(OEP)Cl in PVC/DOS film to a chloride ion solution prepared in pH 4.2 phosphate buffer (absorbance measured at 530 nm).

Table 3

Results obtained for chloride determinations in pharmaceuticals formulations (g chloride/100 ml solution)

Samples	Reference method ^a	Potentiometric determination ^a	R.D. (%) ^b	Optical determination ^a	R.D. (%) ^b	Average results	R.D. (%) ^b
Maia Lab	0.565 ± 0.010	0.574 ± 0.012	1.6	0.560 ± 0.008	−0.9	0.567	0.4
Glucosteril	0.583 ± 0.010	0.567 ± 0.012	−2.7	0.598 ± 0.010	2.6	0.583	0.0
Fresenius	0.579 ± 0.007	0.587 ± 0.011	1.4	0.565 ± 0.009	−2.4	0.576	−0.5
Mustela	0.585 ± 0.004	0.567 ± 0.012	−3.1	0.589 ± 0.012	0.7	0.578	−1.2
Dextran	0.579 ± 0.008	0.557 ± 0.009	−3.8	0.564 ± 0.010	−2.6	0.561	−3.1

^a Average of four determinations ± standard deviation.^b Relative deviation from the reference method.

The optical selectivity coefficients against several anionic species were assessed by the separated solutions method for α values of 0.5 [26]. As shown in Table 2, except for a few anions, the membrane presented a selectivity pattern similar to those for the potentiometric membrane, regarding the lipophilic characteristics of the interfering species. The difference probably can be related to the strength of the ETH ligand and/or the mediator solvent which were used in membrane preparation. The repeatability of the optical detection system was evaluated by processing 10 replicates of chloride solutions with concentrations of $1.00 \times 10^{-2} \text{ mol l}^{-1}$ and $1.00 \times 10^{-1} \text{ mol l}^{-1}$, which provided relative standard deviations of 0.96 and 0.52%, respectively. The analytical frequency was 14 samples per hour. The lifetime of the optode was shorter than of its potentiometric counterpart, it being necessary to replace the membranes after four working days, as they became disrupted.

3.4. Analytical control of pharmaceutical formulations

Chloride determinations were performed in various pharmaceutical preparations by employing the two proposed methods, according to the procedures summarised in Table 1. Furthermore, the determinations of chloride were also carried out by the reference method [17], aimed at evaluating the accuracy of the results obtained by these methods, as shown in Table 3. The application of the Student's *t*-paired test indicated the absence of statistical differences at a confidence level of 95% for the results obtained ($t = 1.160$ for the potentiometric/reference procedures; $t = 0.514$ for the optical/reference procedures). After evaluating the relative deviations achieved in the analysis of the samples, it can be concluded that averaging the values obtained by the two methods provide more accurate results, as random errors are minimised.

4. Conclusions

Sensing membranes based on In(III)octaethyl-porphyrins, besides presenting high selectivity for chloride ions, were of simple preparation and easily coupled to a sequential injection system for analytical purposes. The procedures developed enabled the accomplishment of chloride deter-

mination in pharmaceuticals by two different transduction techniques, saving costs with respect to reagent consumption and sample pre-treatment. The implementation of the two quasi-simultaneous detection systems provides both real-time accuracy assessment and failure detection in large scale analysis.

Acknowledgements

The authors thank Nuno Milhazes for the synthesis and purification of the metalloporphyrin. One of the authors (A.M.P.) wishes to thank the Praxis Program for a Ph.D. grant (Praxis/BD/19793/99). Authors are also grateful to Professor C.H. Collins for manuscript revision and to CAPES (Brazil) and ICCTI (Portugal) for financial support.

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