Ciprofloxacin-imprinted polymeric receptors as ionophores for potentiometric transduction

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ABSTRACT

A 3D-mirror synthetic receptor for ciprofloxacin host–guest interactions and potentiometric transduc- tion is presented. The host cavity was shaped on a polymeric surface assembled with methacrylic acid or 2-vinyl pyridine monomers by radical polymerization. Molecularly imprinted particles were dis- persed in 2-nitrophenyl octyl ether and entrapped in a poly(vinyl chloride) matrix. The sensors exhibited a near-*Nernstian* response in steady state evaluations. Slopes and detection limits ranged from 26.8 to 50.0 mV decade^{-1} and 1.0 × 10^{-5} to 2.7 × 10^{-5} mol L^{-1}, respectively. Good selectivity was observed for trimethoprim, enrofloxacin, tetracycline, cysteine, galactose, hydroxylamine, creatinine, ammonium chloride, sucrose, glucose, sulphamerazine and sulfadiazine. The sensors were successfully applied to the determination of ciprofloxacin concentrations in fish and in pharmaceuticals. The method presentedoffered the advantages of simplicity, accuracy, applicability to colored and turbid samples and automa- tion feasibility, as well as confirming the use of molecularly imprinted polymers as ionophores for organic ion recognition in potentiometric transduction.

Keywords: Ciprofloxacin, Molecularly imprinted sensors Potentiometry, Aquaculture

1. Introduction

Quinolones, broad-spectrum synthetic antibiotics that disrupt bacterial gene replication [1,2], are subdivided into several groups, including the well-known fluoroquinolones. These antimicrobials are fluorinated piperazinyl quinolones, are widely used for the treatment of respiratory tract infections, skin and soft tissue infec- tions, sexually transmitted diseases, urinary tract infections, as well as in sewage treatment plant outlets, streams and in connection to aquaculture [3,4].

Ciprofloxacin (CIPRO) is a fluoroquinolone widely used for veterinary purposes [5]. It is analyzed using various methods, including high performance liquid chromatography [6–8], spec- trophotometry [9–11], capillary zone electrophoresis [12,13], chemiluminescence [14], or micellar liquid chromatography [15]. Thus, a simple and low cost procedure mostly for screening pur- poses would be highly desired.

A simple, alternative method could rely on ion-selective elec- trodes (ISEs) and potentiometric detection. Ion-selective sensors have replaced many wet analytical methods because they offer high precision, fast response, low cost of analysis, good selectivity and high sensitivity [16,17]. Still, the sensing material plays a key role in the sensitivity and selectivity of the electrode. The design of sens- ing materials that are complementary to the size and charge of a particular ion can lead to very selective interactions.

The ionophore, or ion carrier, is the most vital component in a polymeric membrane sensor in terms of selectivity [18]. The bind- ing between the ionophore and the target ion is a molecular-level phenomenon, sensed by an ISE [18]. Ion exchangers and neutral macrocyclic compounds have been employed over the past decades for potentiometric transduction. Until now, only few reports in lit- erature describe the use of molecularly imprinted polymers (MIPs) as potentiometric sensing materials [19–22].

MIPs are synthetic materials tailored with selectivity for a pre- determined ligand [18]. They mimic the action of antibodies and enzymes [23] and can be easily tailored with selectivity for a guest molecule [24]. MIPs hold many advantages over natural receptors, including their stability at extreme pH values and temperatures, high mechanical strength, low cost and reusability.

The present work describes the development of CIPRO MIP-based ISEs. The sensor was synthesized by polymerizing methacrylic acid (MAA) and 2-vinyl pyridine (VPY) functional monomers in the presence of the template molecule (CIPRO) and cross-linking the growing oligomers by ethylene glycol dimethacrylic acid (EGDMA). The sensing materials were dispersed in PVC and plasticized with *o*-nitrophenyl octyl ether (*o*NPOE). The performance characteristics and selectivity of the sensors in batch and flow conditions were evaluated and discussed. The sensors exhibited significantly high sensitivity, stability and selectivity for CIPRO ions over many common ions and were successfully used for

determining CIPRO ions in spiked fish and pharmaceutical prod- ucts.

2. Experimental

2.1. Apparatus

All potential measurements were made by a Crison μpH 2002 decimilivoltammeter (±0.1 mV sensitivity) at room temperature with constant stirring, by means of a Crison micro ST 2038. The output signal in steady state evaluations was transferred to a commutation unit and reconnected to one of six exports, enabling the simultaneous reading of six ISEs. The assembly of the potentiometric cell was as follows: conductive graphite | CIPRO selective membrane | buffered sample solution (HEPES, 1×10^{-2} M, pH 4.0) || electrolyte solution, KCl | AgCl(s) | Ag. The reference electrode was an Orion Ag/AgCl double-junction (Orion 90-02-00). The selective electrode was prepared in conventional or tubular configurations [25] for batch and flow mode evaluations, respectively. Both devices had no internal reference solution and epoxy-graphite was used as the solid contact.

When necessary, the pH was measured by a Crison CWL/S7 com- bined glass electrode connected to a decimilivoltammeter Crison pH meter, GLP 22.

2.2. Reagents and solutions

All chemicals were of analytical grade and de-ionized water (conductivity <0.1 μS cm^{-1}) was employed. CIPRO, potassium *tetrakis*(4-chlorophenyl)borate (TpClPB), *o*NPOE, poly(vinyl chloride) (PVC) of high molecular weight, EGDMA, VPY and MAA were purchased from Fluka. Benzoyl peroxide (BPO), methanol (MeOH) and tetrahydrofuran (THF) were obtained from Riedel-deHäen.

Stock solutions of 0.01 M CIPRO were prepared in water. Less concentrated standards were prepared by suitable dilution in ultra- pure water. The buffer solution used was 0.01 M HEPES (pH ~5.4). The effect of pH was studied by imputing pH variations on 200 mL of a 1.0×10^{-4} M CIPRO solution. The pH of this solution was altered by small additions of either concentrated sulphuric acid or saturated sodium hydroxide solution, freshly prepared. Interference of other chemicals was evaluated for 1.2×10^{-4}, 5.0×10^{-4} and 1.0×10^{-3} M solutions of sodium carbonate, sodium chloride, sodium fluoride, sodium nitrate, bicarbonate and sodium nitrite. All these solutions were prepared in buffer.

2.3. Synthesis of host-tailored polymers

MIP particles were synthesized by placing the template (CIPRO, 0.5 mmol) in a glass tube (14.0 mm i.d.) and adding the functional monomer (3.0 mmol MAA or VPY), the cross-linker (EGDMA, 15.0 mmol) and the radical initiator (BPO, 0.32 mmol), all dissolved in 3 mL MeOH/water (7:3). The mixture was sonicated, degassed with nitrogen for 5 min and cured at 70 °C for 30 min. Non-imprinted polymers (NIP) were also prepared in a similar way by excluding the template from the procedure.

Non-reacted species (excessive reagents or templates) were removed from the polymers by consecutive washout of the parti- cles with methanol/acetic acid (5:1, v/v). The elimination of CIPRO from the MIPs was confirmed by measuring the absorbance of the washout solution at 276 nm. The polymer was then dried at 60 °C under vacuum until constant weight and ground/sieved to particle sizes of 50–150 μm. All polymers (MIP/MAA, NIP/MAA, MIP/VPY, NIP/VPY) were dried at ambient temperature before use.

2.4. Potentiometric sensor

The membrane cocktail was prepared with 200 mg of PVC, 350 mg of plasticizer *o*NPOE and 15 mg of the sensing polymer (Table 1). Some membranes were also added to 7 mg of TpClPB, act- ing as an anionic additive. The mixture was stirred until the PVC was well moistened and dispersed in 3.0 mL THF. The membranes were placed in conductive supports of conventional or tubular shapes. Membranes were dried for 24 h and placed in a 1×10^{-4} M CIPRO solution. The electrodes were kept in this solution when not in use.

2.5. Potentiometric procedures

All potentiometric measurements were carried out at room temperature. The emf values for each electrode were measured in solutions of fixed pH and ionic strength. Increasing concentra- tion levels of CIPRO were obtained by transferring 0.0200–10.0 mL aliquots of 1.0×10^{-2} M aqueous CIPRO solutions to a 100 mL beaker containing 50.0 mL of 1.0×10^{-2} M suitable buffer. Potential readings were recorded after stabilization to ±0.2 mV and the emf was plotted as a function of log CIPRO concentration. Calibration graphs were used for subsequent determination of unknown CIPRO concentrations.

2.6. Binding experiments

Binding experiments were carried out by placing 20.0 mg of MIP-washed particles in contact with 10.0 mL CIPRO solutions ranging 0.04–2 mM. The mixtures were oscillated for 12 h at room temperature and the solid phase was separated by centrifugation (3000 rpm, 10 min). The concentration of free CIPRO in the super- natant was detected by UV spectrophotometry at 276 nm. The amount of CIPRO bound to the polymer was calculated by subtract- ing the concentration of free CIPRO from the initial CIPRO concen- tration. The data obtained were used for a Scatchard analysis.

2.7. Determination of CIPRO

2.7.1. Determination of CIPRO in fish samples

Constant weights of well-ground fish (~2.0 mg) from aquacul- ture origin were transferred to 15 mL tubes. A 10 mL portion of

Table 1
Membrane composition of CIPRO PVC membrane sensors and their potentiometric features in 10^{-2} M HEPES buffer, pH 4.0.

Characteristics	ISE I	ISE II	ISE III	ISE IV	ISE V	ISE VI
Membrane materials						
Sensing polymer	MIP/MAA	MIP/MAA	NIP/MAA	MIP/VPY	MIP/VPY	NIP/VPY
Additive	TpClPB	–	–	–	TpClPB	–
Slope, mV decade^{-1}	46.6 ± 1.0	50.0 ± 0.1	33.6 ± 1.0	34.5 ± 0.2	32.3 ± 1.1	26.8 ± 0.4
Correlation coefficient, r^2 (n = 5)	0.998	0.990	0.999	0.994	0.991	0.993
Detection limit, mol L^{-1}	1.0×10^{-5}	1.0×10^{-5}	2.0×10^{-5}	2.7×10^{-5}	2.7×10^{-5}	2.0×10^{-5}
Lower limit of linear range, mol L^{-1}	2.0×10^{-5}	2.0×10^{-5}	5.0×10^{-5}	6.0×10^{-5}	6.0×10^{-5}	7.0×10^{-5}
Response time, s	<15	<15	<15	<15	<15	<15
Standard deviation, σ_y (mV)	2.1	2.4	2.0	1.5	3.4	0.7
Repeatability, Cv_w(%)	0.08	0.17	0.16	0.00	0.17	0.10

0.01 M HEPES buffer was added and thoroughly mixed with the fish sample. A sonication period of 5 min was allowed to ensure convenient extraction of the analyte. A supernatant liquid was obtained by centrifugation at 1000 rpm and transferred into a 25 mL volumetric flask after filtration. Analytical measurements were conducted over this solution after completing the flask to final volume with buffer.

2.7.2. Determination of CIPRO in tablets

Potentiometric analysis was conducted on oral dosage forms of pharmaceutical preparations, commercially available as Bluepharma®, with a labeled amount of 250 mg CIPRO/tablet. Two tablets were ground and a representative amount of powder was transferred to a 50 ml calibrated flask. The powder was dissolved in water after sonication for 10 min. A 1.0 ml aliquot of the clear supernatant was diluted with 1.0×10^{-2} M HEPES solution with a pH of 4.0 in 100 ml measuring flask. A 10-ml aliquot of the previ- ous solution was placed in the potentiometric cell for analytical measurement.

3. Results and discussions

CIPRO has both amino and carboxylic acid groups (Fig. 1), mak- ing it an ideal compound to interact with acidic or basic monomers such as MAA or VPY, respectively. Non-covalent bonds between CIPRO and polymer cavities were established in this condition, allowing fast and reversible host–guest interactions.

3.1. Binding characteristic of the MIP

In liquid phase applications of MIPs, a molecule in solution inter- acts with the binding sites of the solid adsorbent. The free ligand concentration in the liquid-phase is constant after equilibrium is reached and is easily quantified by an adsorption isotherm. This was determined by plotting the binding capacity (Q) against the free ligand. Q was calculated according to following equation:

$$Q = \frac{\mu\text{mol (CIPRO bound)}}{\text{g (MIP)}} = \frac{(C_i - C_f)V_s \times 1000}{M_{MIP}} \quad (1)$$

where Q is the binding capacity of MIPs (μmol/g), C_i is the initial CIPRO concentration (μmol/ml), C_f is the final CIPRO concentration (μmol/ml), V_s is the volume of solution tested (ml) and M_{MIP} is the mass of dried polymer (mg). The adsorption isotherms obtained after keeping varying concentrations of CIPRO with the synthe- sized particles for several hours under continuous stirring were plotted in Fig. 2A_1 and A_2. In general, adsorption data showed

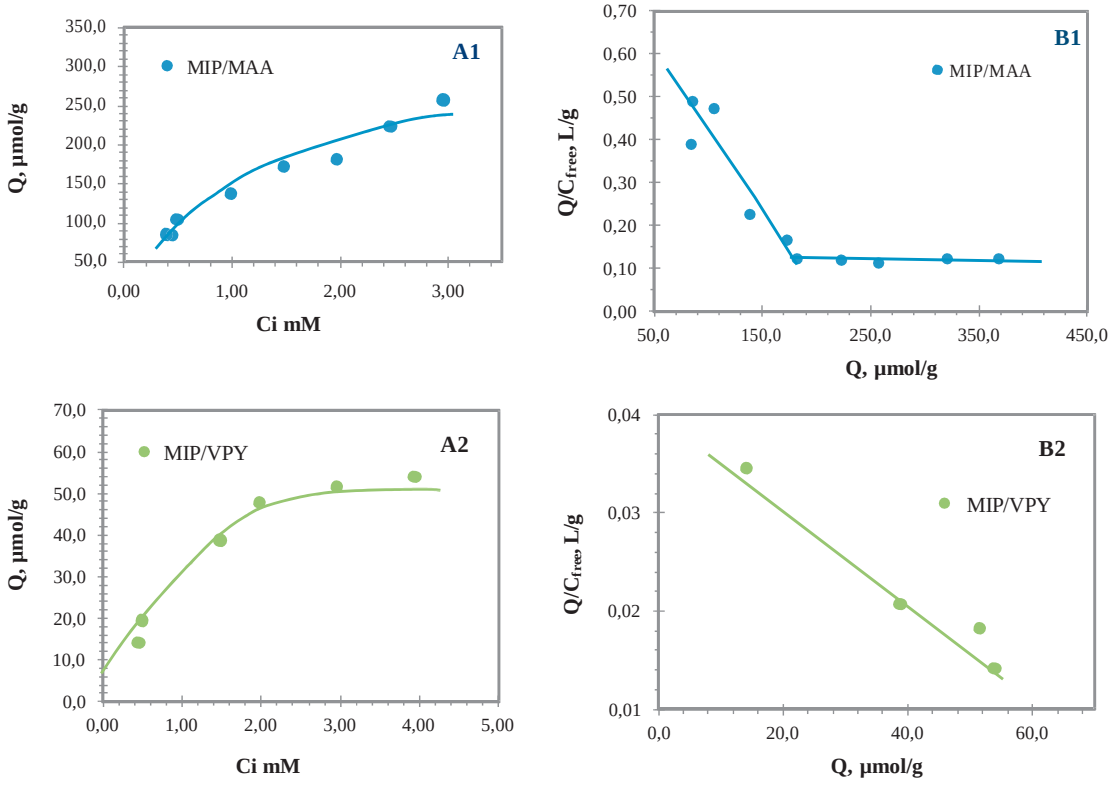

Fig. 2. Binding isotherm (A_1 and A_2) and Scatchard plot (B_1 and B_2) for ENR/MAA imprinted polymer. Q is the amount of CIPRO bond to 20.0 mg of polymer; t = 25 °C; V = 8.00 mL; binding time: 20 h.

that the binding capacity of MIPs increased with increasing initial concentrations of ligand, leading to saturation at higher concentra- tions. MIP/MAA particles required higher concentrations than the MIP/VPY polymer, indicating that MAA-based polymers displayed a higher affinity for CIPRO.

The binding parameters of the MIP/CIPRO binding were calcu- lated by Scatchard analysis, with the following equation:

$$\frac{Q}{C_{free}} = \frac{Q_{max} - Q}{K_d} \quad (2)$$

where Q is the binding capacity, C_{free} is the free analytical concentration at equilibrium (μmol/L), Q_{max} is the maximum apparent binding capacity and K_d is the dissociation constant at binding site. The equilibrium dissociation constant was calculated from the slopes and the apparent maximum number of binding sites from the *y*-intercepts in the linear plot of Q/C_{free} vs. Q.

As shown in Fig. 2B$_1$, the Scatchard plot for MAA as a monomer was not linear in all CIPRO concentration ranges, suggesting that the binding sites in the MIP were not uniform. The plot shows two distinct sections that can be regarded as straight lines, revealing two classes of binding sites in the MIP. The equilibrium dissocia- tion constant K_{d1} and the apparent maximum amount Q_{max1} for the higher affinity binding sites were calculated to be 287 μM and 217 μmol g^{-1} for the dry polymer. Using the same treatment, K_{d2} and Q_{max2} for the lower affinity binding sites were calculated to be 2329 μM and 1106 μmol g^{-1}.

In contrast, when VPY was used as a monomer, the Scatchard plot was linear in all concentration ranges, suggesting that the binding sites were homogeneous and of one type. The equilib- rium dissociation constant K_{d1} and the apparent maximum amount Q_{max1} for the higher affinity binding sites were calculated to be 2100 μM and 95 μmol g^{-1} for the dry polymer.

3.2. Performance of the sensors

CIPRO sensors contained either MIP or NIP particles as electroactive materials and were incorporated in a PVC membrane that was plasticized with *o*NPOE. Characterization of their primary analytical features followed IUPAC recommendations [26] and the corresponding results are shown in Table 1.

CIPRO sensors based in MIP particles displayed different sensitivity and detection limits (Fig. 3). The sensors prepared with MAA and VPY, showed linear responses starting at 2.0×10^{-5} and 7.0×10^{-5} M CIPRO, cationic slopes of 50.0 and 34.5 mV $decade^{-1}$ and detection limits of 3.31 and 23.19 μg mL^{-1}, respectively. The corresponding NIP particles displayed a linear response after 5.0×10^{-5} and 2.7×10^{-5} M, cationic slopes of 33.6 and 26.8 mV $decade^{-1}$ and detection limits of 6.62 and 19.89 μg mL^{-1}, respectively. In general terms, near-*Nernstian* slopes were obtained only with MIP/MAA sensing membranes, thus confirming the pre- vious binding data. NIP-based sensors showed a poor analytical performance.

The MIP-based sensors were also added to TpClPB, an anionic lipophilic compound (Table 1). The procedure generally reduced the anionic interference and lowered the electrical resistance of the membranes [27]. Sensors based in MIP/MAA and VPY showed the linear response ranges of 2.0×10^{-5} and 2.7×10^{-5} M, 3.31 and 23.19 μg mL^{-1} detection limits and *Nernstian* responses of 46.6 and 32.3 mV $decade^{-1}$, respectively. When compared to the corresponding sensors without additive, no significant improvement in terms of slope and lower limit of linear range were observed for MAA and VPY sensors (Fig. 3). This result appeared to indicate that the MIP particles acted as charged carriers, not requiring the presence of additional ionic sites.

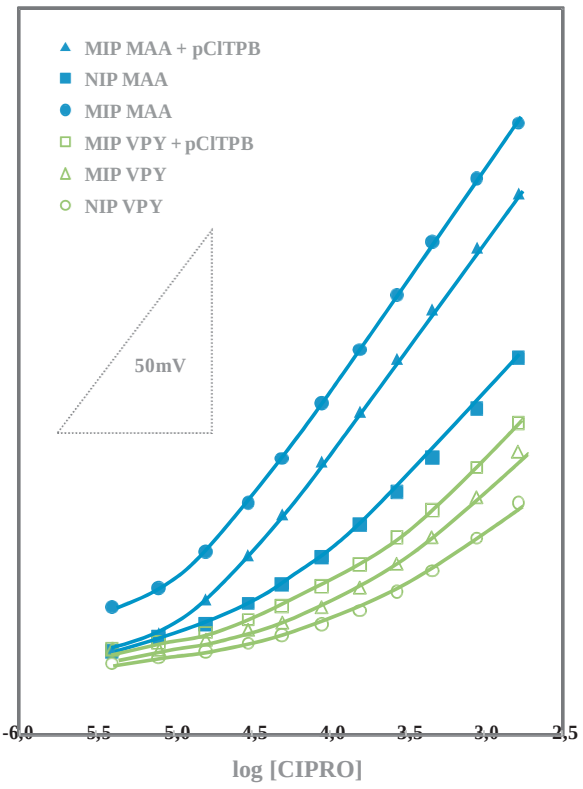

Fig. 3. Potentiometric response of CIPRO PVC membrane sensors under static mode of operation.

3.3. Response time and lifetime

The time required to achieve a steady potential response (±3 mV) was evaluated within 10^{-6}–10^{-4} M and for a rapid 10- fold increase. The response time increased for increasing CIPRO concentrations, but was always below 15 s. Replicate calibrations in consecutive days for each sensor showed a low potential drift (below 10 mV) and long-term stability. In this period, the sensors remained in 10^{-3} M CIPRO pH 4.0 solution when not in use.

In general, the primary analytical features of the sensors were reproducible within ±3% of their original values over a period of at least 7 weeks. Detection limits, response times, linear ranges and calibration slopes were regarded for this purpose.

3.4. Effect of pH

CIPRO contains two ionizable functional groups: a carboxylic group (pK_1 = 5.90) and a basic piperazinyl group (pK_2 = 8.89). Depending on the pH, it can exist in four forms: cationic (C), neutral non-ionized (N), zwitterionic (Z) and anionic (A) (Fig. 1) [28,29]. In strong acidic conditions, only the 7-piperazinyl group is positively charged, while in strongly basic medium, only the 3-carboxylic group is negatively charged. The amphiprotic form exists in neutral pH values. However, other work reports the additional protona- tion of the amine groups, stating that at pH 2, the major species of ciprofloxacin is $CIPRO^{3+}$, while at pH 4–5 $CIPRO^{2+}$ is predom- inant; at pH from 6 to 7 $CIPRO^{+}$, $CIPRO^{2+}$ and CIPRO species are present, while at pH >7 the major species are anionic. Thus, the pH of the measuring solution must play an important role on the potentiometric response.

The pH effect was investigated by following the variation in potential with change in pH by addition of very small amounts

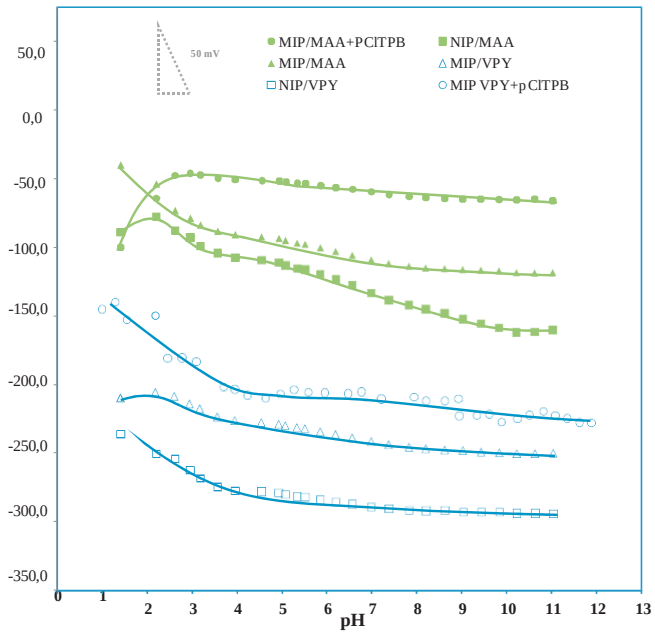

Fig. 4. Influence of the pH on the potential of CIPRO membranes selective electrodes.

of concentrated hydrochloric acid or saturated sodium hydroxide solutions. The emf of a standard solution of 1.0×10^{-4} M CIPRO was plotted against pH (Fig. 4). The results indicated that the electrode did not respond to pH changes within 3.0–4.5 and after pH 9. In these pH ranges, the emf variations were always below ±10 mV. Generally, acidic medium potentials were constant and above pH 4.5, they started to decrease slightly. Only NIP electrodes behaved differently and appeared to be more affected by pH; they displayed a constant potential decay for higher pH values.

In general, the narrow pH operational ranges were a result of the several isoforms of CIPRO, presenting different charges and concen-tration levels for each specific pH. In terms of analytical response, this was confirmed by plotting at least three calibration curves for all electrodes in buffer solutions with pH values of 3.0, 4.0, 4.5, 5.0 and 6. In general, MAA-based sensors showed the best analytical performance, with the higher slopes and lower detection limits. For these electrodes, an average slope of approximately 50 mV/decade decreased for pH >4.0 down to 40 mV/decade at pH 6. The lower limit of the linear range was approximately $1–2 \times 10^{-5}$ mol/L for pH values of 3 and 4, but increased after to $1–3 \times 10^{-5}$ M. The VPY-based sensors showed a similar behavior but presented signifi- cantly worse analytical features. The average slopes ranged from 13 to 45 mV/decade at pH 3 and changed to 27–35 mV/decade at pH 4. For higher pH values, the NIP/VPY electrodes started responding as a negatively charged species and the other units displayed average slopes of about 25 mV/decade. Due to the existence of a zwitteri- onic form or to a doubly charged species, the electrodes could not operate in near-neutral pH conditions. A pH of 4 appeared to be the best choice for the reported electrodes.

3.5. Sensor selectivity

The selectivity profile of each sensor was evaluated by calculating potentiometric selectivity coefficients ($K^{POT}_{CIPRO,J}$), assessed by the separate solution method (SSM) and the mixed solution method (MSM) [30]. The methods indicated the degree of prefer- ential interaction for CIPRO over foreign species that are common in biological and food samples, such as other fluoroquinolones used in aquaculture, namely enrofloxacin (ENR), or other antibiotics such as tetracycline (Tc^+), sulfamerazine (SMZ), sulfathiazole (STZ) and trimethoprim (TMP). Glucose (Glu), hydroxylamine (HDXL), sucrose (SAC), ammonium chloride (NH_4Cl) and creatinine ($Crea^+$) were also included as possible interfering species.

The selectivity coefficients for SSM and MSM were indicated in Tables 2 and 3 (expressed in log $K^{POT}_{CPZ^+,J^{z+}}$) and calculated with the following equations:

$$K^{POT}_{CIP,J} = a_{CIP}^{1-(1/Z_J)} e^{(E_J - E_{CIP})/S} \quad (3)$$

$$K^{POT}_{CIP,J} = \frac{a_{CIP}}{a_J^{Z_{CIP}/Z_J}} \quad (4)$$

In Eq. (3), E_{CIP} is the electrode potential in a 1.0×10^{-3} M CIPRO solution, E_J the potential of the electrode facing a 1.0×10^{-4} M concentration in interfering species J^{z+} of charge Z and S the practical slope calculated after the calibration experiments. In Eq. (4), a_J is 1.0×10^{-4} M of interfering species, Z the ionic charges of main and interfering ions and a_{CIP} the intersection of the extrapolated linear portions of the plot emf vs. the logarithm of CIPRO concentration. In general, MSM and SSM produced different results in terms of rel- ative order of selectivity and in log $K^{POT}_{CIP,J}$ absolute values for each interfering species, with the MSM indicating better selectivity for all electrodes.

Using the MSM method, MIP-based sensors displayed the low- est log K^{POT} values and were more selective than the NIP sensors (Table 2). Sensors with TpClPB displayed higher log K^{POT} than the corresponding ones without this compound, suggesting that this membrane component hindered the selectivity of the electrodes. MAA- and VPY-based sensors showed a similar behavior.

Generally, the SSM method offered higher K^{POT} values than the previous method (Table 3). According to the result obtained,

Table 2
Potentiometric selectivity coefficients (log K^{POT}) with mixed solutions method (MSM) of CIPRO membrane based sensors, in 0.01 M HEPES buffer of pH 4.0.

Interfering species	log K^{POT}, MSM					
	ISE I	ISE II	ISE III	ISE IV	ISE V	ISE VI
Trimethoprim	−2.46	−2.76	−0.66	−0.96	−0.96	−0.76
Enrofloxacin	−1.91	−1.91	−1.96	−1.96	−0.98	−1.34
Tetracycline	−3.06	−2.56	−3.36	−3.54	−3.21	−3.16
Cysteine	−2.46	−2.38	−2.86	−2.51	−3.76	−2.06
Galactose	−2.34	−2.45	−3.36	−2.73	−3.46	−2.41
Hydroxylamine	−2.56	−2.36	−2.76	−2.71	−2.96	−2.56
Creatinine	−2.58	−2.36	−2.06	−2.66	−1.71	−2.36
Ammonium chloride	−2.81	−2.51	−3.24	−2.46	−3.06	−2.61
Sucrose	−2.76	−2.56	−2.39	−2.66	−3.61	−2.06
Glucose	−2.74	−2.56	−3.16	−2.66	−2.76	−2.46
Sulphamerazine	−2.61	−2.26	−2.86	−2.81	−2.96	−0.66
Sulfadiazine	−2.78	−2.16	−3.85	−2.61	−3.31	−2.46

Table 3
Potentiometric selectivity coefficients ($\log K^{POT}$) with separated solution method (SSM) of CIPRO membrane based sensors, in 0.01 M HEPES buffer of pH 4.0.

Interfering species	$\log K^{POT}$, SSM					
	ISE I	ISE II	ISE III	ISE IV	ISE V	ISE VI
Trimethoprim	1.02	1.58	1.15	2.14	1.90	−1.74
Enrofloxacin	0.47	−0.90	0.89	0.89	−1.09	0.17
Tetracycline	−1.35	−0.73	−1.34	−1.39	−0.97	−0.04
Cysteine	−1.66	−0.95	−1.72	−1.67	−1.12	0.11
Galactose	−2.31	−1.86	−2.09	1.91	−1.79	0.95
Hydroxylamine	−1.76	−0.98	−1.95	−2.05	−1.23	0.19
Creatinine	−2.27	−1.94	−2.17	−1.77	−2.07	0.56
Ammonium chloride	−2.63	−2.28	−2.44	−3.83	−1.88	0.84
Sucrose	−1.77	−1.01	−1.94	−1.25	−0.94	0.00
Glucose	−1.93	−0.63	−0.93	−0.76	−0.67	0.00
Sulphamerazine	−1.62	−0.09	0.00	1.04	−0.51	0.00
Sulfadiazine	−1.96	−2.32	−2.26	−0.62	−2.90	−0.27

Table 4
Batch potentiometric determination of CIPRO ions in spiked fish samples or drugs using MIP/MAA based membrane sensor with additive.

Sample	CIPRO (mgL^{-1})		RSD (%)	Recovery (%)
	Added	Found		
Fish 1	113.8	111.6 ± 5.6	5.0	98.0 ± 5.44
Fish 2	172.7	182.0 ± 0.57	0.7	105.4 ± 0.42
Fish 3	26.0	25.4 ± 1.37	5.4	97.7 ± 5.28
Fish 4	64.1	63.6 ± 3.04	4.8	99.2 ± 5.54
Drug 1	250.0	244.5 ± 28.6	11.7	97.8 ± 11.5

the ISEs suffered strongly, interfering with some compounds because the positive values of log K^{POT} indicated that the electrode responded more selectively to some interfering ions than to the cationic form of CIPRO. However, the MSM appeared to be more reliable because it is similar to real sample conditions.

3.6. Analytical application

The method was applied to determine the CIPRO concentra- tion in samples typically produced in aquaculture in the following fish: salmon, trout and sea bass. Fish meat was ground and spiked with 26.0–172.7 μg mL^{-1} of CIPRO. The analytical data obtained are shown in Table 4 and represent the mean of at least 3 inde- pendent determinations. A strong agreement was found between spiked and detected amounts of CIPRO. The potentiometric set of results showed recoveries ranging from 97.7 to 105.4%, which cor- responded to relative errors within −2.3 and +5.6%. They were also precise, with relative standard deviations always below 5%. The Student's *t*-test confirmed that there were no significant differ- ences between the means of added amounts and potentiometric sets of results (Table 4). The *p* value was 0.46, below the critical value (2.09).

Bluepharma® tablets with a labeled amount of 250 mg CIPRO were also analyzed by direct potentiometric analysis. A good agreement was also found between theoretical and experimental amounts of CIPRO with recoveries of 97.8% (Table 4). The Student's *t*-test confirmed that there were no significant differences between the means of the theoretical amount and potentiometric results (Table 4), with a *p* value of 0.88 and below the critical *t* (3.18).

4. Conclusions

The molecular imprinting technique was employed to produce CIPRO host-tailored sensors for potentiometric transduction. Bind- ing data indicated that MAA-based sensors showed a higher affinity for the template than VPY-based particles. These results were confirmed by the comparison of the potentiometric response of such devices. MAA sensors offered the best potentiometric analyt- ical features and high analytical suitability, capable of producing accurate and precise analytical data and presented a good abil- ity to discriminate CIPRO from other co-existing compounds in real samples. Advantages of these sensors include the simplicity in designing, short measurement time, good precision, high accu- racy, high analytical throughput, low limit of detection and good selectivity. Overall, the proposed method was suitable for the rou- tine screening of CIPRO because of the simple, precise, accuracy and low cost regarding reagent consumption and the equipment involved.

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