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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 transduction 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 dispersed 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 presented offered the advantages of simplicity, accuracy, applicability to colored and turbid samples and automation feasibility, as well as confirming the use of molecularly imprinted polymers as ionophores for organic ion recognition in potentiometric transduction.

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 infections, 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], spectrophotometry [9–11], capillary zone electrophoresis [12,13], chemiluminescence [14], or micellar liquid chromatography [15]. Thus, a simple and low cost procedure mostly for screening purposes would be highly desired.

A simple, alternative method could rely on ion-selective electrodes (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 sensing 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 binding 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 literature describe the use of molecularly imprinted polymers (MIPs) as potentiometric sensing materials [19–22].

MIPs are synthetic materials tailored with selectivity for a predetermined 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

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determining CIPRO ions in spiked fish and pharmaceutical products.

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 combined 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 ultrapure 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 particles 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, acting 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 concentration 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 supernatant was detected by UV spectrophotometry at 276 nm. The amount of CIPRO bound to the polymer was calculated by subtracting the concentration of free CIPRO from the initial CIPRO concentration. 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 aquaculture 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_v (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