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Sulfadiazine-selective determination in aquaculture environment: Selective potentiometric transduction by neutral or charged ionophores

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ABSTRACT

Solid-contact sensors for the selective screening of sulfadiazine (SDZ) in aquaculture waters are reported. Sensor surfaces were made from PVC membranes doped with tetraphenylporphyrin-manganese(III) chloride, α-cyclodextrin, β-cyclodextrin, or γ-cyclodextrin ionophores that were dispersed in plasticizer. Some membranes also presented a positive or a negatively charged additive. Phorphyrin-based sensors relied on a charged carrier mechanism. They exhibited a near-*Nernstian* response with slopes of 52 mV decade^{-1} and detection limits of 3.91×10^{-5} mol L^{-1}. The addition of cationic lipophilic compounds to the membrane originated Nernstian behaviours, with slopes ranging 59.7–62.0 mV decade^{-1} and wider linear ranges. Cyclodextrin-based sensors acted as neutral carriers. In general, sensors with positively charged additives showed an improved potentiometric performance when compared to those without additive. Some SDZ selective membranes displayed higher slopes and extended linear concentration ranges with an increasing amount of additive (always <100% ionophore). The sensors were independent from the pH of test solutions within 2–7. The sensors displayed fast response, always <15 s. In general, a good discriminating ability was found in real sample environment. The sensors were successfully applied to the fast screening of SDZ in real waters samples from aquaculture fish farms. The method offered the advantages of simplicity, accuracy, and automation feasibility. The sensing membrane may contribute to the development of small devices allowing in locus measurements of sulfadiazine or parent-drugs.

1. Introduction

Aquaculture is the farming of aquatic organisms, practiced for centuries. This practice is today accompanied by the introduction of many chemical substances for therapeutic or prophylactic purposes. Particular attention is given to the use of antibiotics since they enter the environment once leached from faeces and/or uneaten antibiotic feed. Most of the antibiotics given in feed are exported to the surrounding environment and accumulate in the sediment. Thus, the routine analytical control of aquaculture waters should contribute to decrease this environmental contamination.

Sulfonamides are among the most used antibiotics in European countries with contributions between 11 and 24% [1]. They have been used in human medicine against a wide variety of microbes, being their current use primarily in the treatment of urinary tract infections and in farm animal feedstuff and fish cultures as veterinary drugs [2]. Consequently, sulfonamides may reach the foodchain by means of meat or water contamination by transport through soil or surface runoff and uptake by plants [3,4]. Besides possible adverse effects on microorganisms, the major risk of introducing antibiotics into the environment is the development and spreading of resistant pathogens [4].

Sulfonamides are *N*-derivatives of 4-aminobenzenesulphonamide, a large group of synthetic antibacterial compounds. They inhibit the conversion of *p*-aminobenzoic acid, interrupting bacterial use of this compound in the synthesis of folic acid and ultimately of purine and DNA. Due to resistance records in formerly susceptible microorganisms, only a few sulpha drugs are used today, among which sulfadiazine (SDZ, Fig. 1).

The analytical control of SDZ is required in several kinds of samples, such as commercial drugs, and biological and food samples. Ideally, this could be achieved by non-destructive and highly selective/sensitive measurements, such as those employing potentiometric sensors. These have found vast applications in many fields of analysis [5–7]. They offer high precision and rapidity, low cost of analysis, enhanced selectivity and sensitivity over a wide range of concentrations [8,9]. In addition, they are easy to construct and manipulate and no sample pretreatment is needed before the

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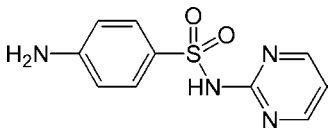

Fig. 1. Chemical structure of SDZ.

analysis itself. Short response times, in the order of seconds, make them appropriate devices for process control and screening analysis [8,9].

Ion-selective membrane sensors, as their name implies, are based on membranes that enable the selective recognition of a specific ion by transferring it (selectively) across the interface between the sample and membrane phase and generating a potential difference [10]. This electrochemical signal is a measure of the activity of that ion. Many mechanisms have been suggested for the selective recognition of different ions [11], most of which mention the selective complexation between the target ion (guest) and a specific carrier (host). This carrier is incorporated in the membrane of the sensor, in order to create the desired selectivity. In general, the overall selectivity of the host–guest interaction depends on several factors such as: (i) the size of the cavity of the host that should be large enough to accommodate the guest species (as the complexation happens, the hydration shell of the target species is removed and substituted by the donor atoms of the host or ligand); (ii) the number of donor atoms in the ligand should be sufficient, to match the coordination number of the target species ; (iii) the flexibility of holding of donor atoms by the host backbone must be limited, so that their positions are suitable to match the shape of the coordination sphere of the target species [12].

An obvious binding strategy for anionic species such as SDZ is to use a positively charged host molecule. However, electrostatic interactions are non-directional and all anions may bind to cations and form either a solvent-separated or contact-ion pair. Anions also bind neutral receptors when there is a difference in electrostatic charge [13]. Thus, neutral or charged carriers, such as cyclodextrin (CD) or porphyrin (PPHR) derivatives may be employed for the potentiometric transduction of SDZ.

CDs are the most widely used receptors in host–guest inclusion chemistry [13,14]. They are a family of cyclic oligosaccharides with a variable number of D-glucopyranoside units linked by 1,4-glycosidic bonds. CDs possess a cage-like supramolecular structure that is generally represented as a cylindrical funnel with an upper (wide) and lower (narrow) rim. The upper rim consists of the secondary hydroxyl groups and the lower of primary hydroxyl groups [13]. It allows carrying out chemical reactions involving "host–guest" electrostatic interactions of intramolecular nature. No covalent bonds are formed or broken in this context. The main driving force of complex formation is the release of enthalpy-rich water molecules from the cavity; water molecules are displaced by more hydrophobic guest molecules present in the solution to attain an apolar–apolar association, resulting in a more stable lower energy state [14].

The PPHR molecule contains four pyrrole rings linked via methine (=CH–) bridges and exhibits aromatic character. The PPHR nucleus is a tetradentate ligand in which there is space available for a coordinated metal. When coordination occurs, two protons are removed from the pyrrole nitrogen atoms, leaving two negative charges [15]. PPHR complexes with transition metal ions are very stable and are often used to construct structures in supramolecular chemistry, taking advantage of the Lewis acidity of the coordinated metal [16]. Several metalloporphyrins have been employed as electroactive components in the membrane of potentiometric sensors, and their response to anions has been interpreted by a dissociation ion-exchange mechanism or metal–ligand interaction mechanism [16–20].

In the presented work, new potentiometric sensors are proposed for SDZ by doping PVC membranes with neutral (CD) or charged ionophores (PPHR) that selectively and reversibly form complexes with the analyte. In addition, it is well known that the nature/amount of ionophore and additives in the selective membrane affect the analytical performance of a potentiometric sensor. A literature survey showed that the usual range of composition in the preparation of PVC matrix membrane sensor was 1–7% ionophore, 28–33% PVC (internal matrix), 60–69% plasticizer (solvent) and 0.03–2% lipophylic additive [21]. Hence, in this work, SDZ sensors were prepared in a similar way, having CD or PPHR based different electroactive materials and different additives in different amounts. Only *o*-nitrophenyl octyl ether (*o*-NPOE) was used as plasticizer, according to a previous study on porphyrin-based sensors [22]. The response behavior of the corresponding sensors is reported herein.

2. Experimental

2.1. Reagents and solutions

All reagents were of analytical grade without further purification. SDZ, tetraoctylammonium bromide (TOABr), dimethyldioctadecylammonium bromide (DDABr), tetrakis (4-chlorophenyl) borate (KTpClPB), tetrahydrofuran (THF), alpha, beta or gamma-CDs (CD), *meso*-tetraphenylporphyrin manganese (III) chloride complex (Mn^{III}TPPCl) and high relative molecular weight PVC were purchased from Sigma–Aldrich. De-ionized water (conductivity <0.1 $\mu S\,cm^{-1}$) was employed in all experiments.

Stock solutions of SDZ 0.01 mol L^{-1} were prepared in water. Less concentrated SDZ standards were prepared by suitable dilution in ultra-pure water. Buffer solutions were 0.01 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES, pH ~ 5.4).

The effect of pH was studied by imputing pH variations on 200 mL of a SDZ solution 1.0×10^{-3} mol L^{-1}. The pH of this solution was altered by little 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} mol L^{-1} solutions of sodium carbonate, sodium chloride, sodium fluoride, sodium nitrate, bicarbonate and sodium nitrite. All these solutions were prepared in buffer.

2.2. Apparatus

Measurements were carried out with the electrochemical cell AgCl(s)/Ag double junction reference electrode/test solution/SDZ selective membrane, graphite-epoxy. An Orion, 90-00-29, double-junction electrode was used as reference. Potential differences between indicator and reference electrodes were measured by means of a Crison® mpH 2002 decimilivoltammeter. The analytical output signal was transferred to a commutation point with six ways out, enabling the reading of six sensors dipped in the same solution. Each way presented an electrical antenna connector that provided suitable adaptation to each sensor.

The pH was measured by a Crison CWL/S7 combined glass electrode connected to a Crison decimilivoltammeter, pH meter, GLP 22. Spectrophotometric assays were carried out on a Shimadzu Pharmaspec UV-1700.

2.3. Preparation of the SDZ sensor

The sensors used an epoxy-graphite matrix as solid contact [9]. This matrix was prepared by mixing Araldit/Hardener with graphite powder in a 1:1 (w/w) ratio and placed before dry on

Table 1
Membrane composition of SDZ sensors.

ISE	Membrane composition				Slope (mV decade^{-1})	R^2 (n = 4)	LOD (mol L^{-1})	LLLR (mol L^{-1})	Response time (s)
	Active ingredient	Plasticizer	Additive	Weight (mg)					
1	Mn^{III}TPPCl	*o*-NPOE	–	5.5:370	51.70 ± 2.10	0.9946	3.88×10^{-5}	1.28×10^{-4}	<15
2	Mn^{III}TPPCl	*o*-NPOE	TOABr	5.5:370:1.1	58.66 ± 0.95	0.9933	3.43×10^{-5}	1.13×10^{-4}	<15
3	Mn^{III}TPPCl	*o*-NPOE	TOABr	5.5:370:2.1	60.23 ± 1.20	0.9956	2.55×10^{-5}	8.41×10^{-5}	<15
4	Mn^{III}TPPCl	*o*-NPOE	TOABr	5.5:370:4.3	61.42 ± 0.81	0.9956	2.99×10^{-5}	9.87×10^{-5}	<15
5	Mn^{III}TPPCl	*o*-NPOE	DDABr	5.5:370:1.2	59.11 ± 2.26	0.9938	2.10×10^{-5}	6.94×10^{-5}	<15
6	Mn^{III}TPPCl	*o*-NPOE	DDABr	5.5:370:2.4	62.02 ± 2.66	0.9940	2.10×10^{-5}	6.94×10^{-5}	<15
7	Mn^{III}TPPCl	*o*-NPOE	DDABr	5.5:370:4.9	61.89 ± 2.85	0.9953	2.10×10^{-5}	6.94×10^{-5}	<15
8	Mn^{III}TPPCl	*o*-NPOE	KTpClPB	5.5:370:1.0	26.57 ± 0.68	0.9948	2.10×10^{-5}	6.94×10^{-5}	<15
9	Mn^{III}TPPCl	*o*-NPOE	KTpClPB	5.5:370:1.9	36.30 ± 2.92	0.9932	2.55×10^{-5}	8.41×10^{-5}	<15
10	Mn^{III}TPPCl	*o*-NPOE	KTpClPB	5.5:370:3.9	43.40 ± 0.92	0.9966	2.55×10^{-5}	8.41×10^{-5}	<15
11	α-CD	*o*-NPOE	–	5.5:370	–				
12	α-CD	*o*-NPOE	TOABr	5.5:370:0.8	47.56 ± 0.66	0.9934	3.91×10^{-5}	1.29×10^{-4}	<15
13	α-CD	*o*-NPOE	TOABr	5.5:370:1.5	54.85 ± 0.51	0.9979	2.56×10^{-5}	8.46×10^{-5}	<15
14	α-CD	*o*-NPOE	TOABr	5.5:370:3.1	53.27 ± 1.93	0.9946	1.88×10^{-5}	6.21×10^{-5}	<15
15	α-CD	*o*-NPOE	DDABr	5.5:370:0.9	52.71 ± 0.82	0.9948	4.95×10^{-5}	1.63×10^{-4}	<15
16	α-CD	*o*-NPOE	DDABr	5.5:370:1.8	59.30 ± 3.36	0.9946	1.20×10^{-5}	3.97×10^{-5}	<15
17	α-CD	*o*-NPOE	DDABr	5.5:370:3.5	64.66 ± 1.03	0.9940	1.80×10^{-5}	5.94×10^{-5}	<15
18	β-CD	*o*-NPOE	–	5.5:370	–				
19	β-CD	*o*-NPOE	TOABr	5.5:370:0.6	49.79 ± 1.61	0.9935	3.43×10^{-5}	1.13×10^{-4}	<15
20	β-CD	*o*-NPOE	TOABr	5.5:370:1.3	57.17 ± 1.49	0.9960	1.87×10^{-5}	6.19×10^{-5}	<15
21	β-CD	*o*-NPOE	TOABr	5.5:370:2.6	65.31 ± 1.07	0.9957	1.21×10^{-5}	3.98×10^{-5}	<15
22	β-CD	*o*-NPOE	DDABr	5.5:370:0.7	59.13 ± 0.27	0.9937	3.91×10^{-5}	1.29×10^{-4}	<15
23	β-CD	*o*-NPOE	DDABr	5.5:370:1.5	60.47 ± 1.56	0.9942	4.71×10^{-5}	1.56×10^{-4}	<15
24	β-CD	*o*-NPOE	DDABr	5.5:370:3.1	59.08 ± 0.90	0.9949	2.71×10^{-5}	8.94×10^{-5}	<15
25	γ-CD	*o*-NPOE	–	5.5:370	–				
26	γ-CD	*o*-NPOE	TOABr	5.5:370:0.6	52.10 ± 1.91	0.9963	3.31×10^{-5}	1.09×10^{-4}	<15
27	γ-CD	*o*-NPOE	TOABr	5.5:370:1.2	64.96 ± 1.35	0.9960	1.21×10^{-5}	3.99×10^{-5}	<15
28	γ-CD	*o*-NPOE	TOABr	5.5:370:2.3	65.16 ± 1.28	0.9952	1.21×10^{-5}	3.99×10^{-5}	<15
29	γ-CD	*o*-NPOE	DDABr	5.5:370:0.7	51.92 ± 1.14	0.9926	4.68×10^{-5}	1.55×10^{-4}	<15
30	γ-CD	*o*-NPOE	DDABr	5.5:370:1.3	56.07 ± 0.91	0.9973	4.95×10^{-5}	1.63×10^{-4}	<15
31	γ-CD	*o*-NPOE	DDABr	5.5:370:2.7	63.70 ± 3.07	0.9975	2.54×10^{-5}	8.40×10^{-5}	<15

top of a cylindrical body. Each PVC membrane was prepared by mixing 5.5 mg of active ingredient with 370 mg of *o*-NPOE acting as plasticizer and variable amount/kind of additive (Table 1). The resulting solution was homogenized in a 180 mg of PVC previously dissolved in about 5 mL THF and casted over the previously indicated a graphite-based conductive supports. Membranes were let dry and conditioned in a 1.0×10^{-3} mol L^{-1} SDZ aqueous solution before use. The sensors were also kept in this solution when not in use.

2.4. Potentiometric procedures

All potentiometric measurements were carried out at room temperature and in solutions of fixed pH and ionic strength. Calibration curves followed the Litre beaker method [21]. All sensors were placed in a convenient support over a magnetic stirrer and immersed in 50.00 mL of HEPES Calibration procedures were made by transferring 0.0200–10.0 mL aliquots of 1.0×10^{-3} mol L^{-1} SDZ aqueous solution into this electrolyte. The potential readings of the stirred SDZ solutions were measured at room temperature and recorded after stabilization to ±0.2 mV. The calibration graph plotted logarithm concentration (mol L^{-1}) against electromotive force (mV).

2.5. Binding studies

The binding between Mn^{III}TPPCl or γ-CD and SDZ was monitored at 475 or 300 nm, respectively. These wavelengths were selected by plotting the spectra of a solution with ionophore and analyte with 1.0×10^{-6} mol L^{-1}. The spectra of single solutions with 1.0×10^{-6} mol L^{-1} of ionophore or SDZ prepared in HEPES/THF (50:50) was also recorded, serving as blank.

The molar ratio between the analyte and the ligand was calculated by adding 300 μL aliquots of a more concentrated SDZ solution to a suitable volume of 1.0×10^{-6} mol L^{-1} ionophore solution. Spectra were recorded for each concentration level of SDZ.

Binding constants were calculated by the Sandwich method. The conductive support of the sensor was first coated with membranes without ionophore and after with membranes carrying the ionophore. The sensors were let stand for 12 h in 1.0×10^{-2} mol L^{-1} SDZ.

2.6. Sample preparation

Samples were collected from several aquaculture units, placed in sweet waters by the north region of Portugal. The waters were collected from the tank itself and from the surroundings, in order to estimate antibiotic dissemination. By the time of collection, there was no previous antibiotic application and these were blank samples. Typically, a dose of 30 mg of active ingredient is administered *per* kg bodyweight of fish daily for a period of 7–10 days. Therefore, the waters were spiked taking into consideration the concentrations expected in each tank after application of a commercial formulation with 333.3 g sulfadiazine *per* kg. The concentration on samples lied within 25 and 250 μg mL^{-1}. The direct potential method was applied to determine SDZ in spiked waters. The analysis was conducted after calibration.

3. Results and discussion

Several parameters, such as binding features, calibration slopes, reproducibility, dynamic linear range, limit of detection, response time, effect of pH and selectivity, were investigated to evaluate the effect of Mn(III) PPHR and CD ionophores on the analytical

performance of SDZ sensors and the need for a charged additive (membrane composition was indicated in Table 1).

3.1. Porphyrin as charged ionophore

Metalloporphyrins are electrically charged carriers in the membrane when uncomplexed and neutral when bounded to anions by axial ligation of the metal center [23]. This ionophore is expected to respond selectively to anionic species such as SDZ if there is a selective coordination of the anion by axial bound to the positively charged metal center.

Thus, the selective interaction between SDZ and the metal center of the PPHR (Mn^{III}) was confirmed by spectrophotometric assays. PPHRs are Naturally Occurring Planar Microcycles with highly sensitive chromogenic properties and their metal chelates generally exhibit characteristic absorption bands in the visible region. The region from 400 to 500 nm, which is called the Soret band, shows the most intensive absorption [16]. Mn^{III}TPPCl provides a Lewis acid binding site for electron donors such as SDZ and the extended *p*-system points out the binding events by means of optical spectrophotometry. Free Mn^{III}TPPCl displayed a sharp absorption band with 475 nm maximum in HEPES/THF (50:50) medium. The increasing addition of SDZ decreased this absorbance, with maximum absorbance at 473 nm, until all PPHR was complexed with the analyte. This was observed when a 1:1 molar ratio of each compound was present (Fig. 2), suggesting that one mole of Mn^{III}TPPCl bound to one mol of SDZ. The average binding constant between SDZ and Mn^{III}TPPCl was 6.40. This value was calculated potentiometrically by means of the Sandwich method.

Mn^{III}TPPCl membrane sensors without additive (Fig. 3, left) exhibited a linear emf response against logarithmic SDZ concentration. They showed near-Nernstian behavior, with average slopes of 52 mV decade^{-1} from 1.3×10^{-4} to 1.0×10^{-2} mol L^{-1}. Although some concerns have been reported in literature about the spontaneous hydroxy-bridged dimer formation of metalloporphyrins in ion-selective membranes originating super-Nernstian response slopes [24–26], this was not observed in the present studies.

3.2. Cyclodextrin as neutral ionophore

CDs are widely known by their ability to form an inclusion complex with hydrophobic guest molecules, because their cavity is hydrophilic outside and hydrophobic inside [13]. Although hydrophilic hydroxyl groups occupy both rims of the cone and the inside of the cavity, they are hydrophobic in character for being covered by C_3–H, C_5–H, and C_6–H and by the ether link oxygen between each glucopyranosyl units [27].

The more or less extent of each inclusion complex is a function of space or thermodynamic aspects. The former one points out that the size of the CD cavity should fit to the size of the guest molecule. The CDs used in this work were α-CD, β-CD and γ-CD. They have six, seven and eight α-(1,4) glycosyl units, respectively, and different cavity sizes. The height of the cavity is equal for all three types, but their diameters vary within 4.7–5.3, 6.0–6.5, and 7.5–8.3 Å, corresponding to estimated volumes of 174, 262, and 427 Å [14]. Based on these dimensions, α-CD can typically accommodate the smaller molecules and γ-CD the larger molecules. To know in anticipate which CD carries the most suitable cavity size to accommodate SDZ is quite difficult because SDZ presents many different conformations and different protonation states varying with the pH. Besides, recent studies have revised the known structure of SDZ, showing that SDZ contains two S–O single bonds instead the typical S=O double bond, bears negative charges over the pyrimidine nitrogen atom and is a very flexible structure [28].

SDZ was studied first as an inclusion guest on the larger CD host cavity. Binding experiments were carried out by analysing the maximum absorbance of SDZ, β-CD and SDZ plus β-CD UV spectra. The addition of SDZ to β-CD increased the 300 nm absorbance. This increase was perceptible after comparing the individual spectra of the two components with that of their mixture. The experimental results suggested that two molecules of SDZ bound to one molecule of CD. Although CDs usually form 1:1 host–guest complexes, 1:2 complexes are also possible [13]. In aqueous solution, the *hydrophobic* cavity is filled with water and the weak nature of these apolar–polar interactions replace it by another less polar guest molecule. The average binding constants between SDZ and α-Cd, β-Cd, and γ-CD were 5.27, 5.46, and 5.22, respectively. As previously indicated, these were calculated by the Sandwich method.

Considering CD membranes without additive (Table 1), none of the CDs in this study were able to provide a suitable emf variation with SDZ concentration (Fig. 3, left). Since the uncharged carriers are neutral when uncomplexed in the membrane and the complexes have the same charge as the analyte ion, the respective membranes require the additional incorporation of lipophilic ions of opposite charge to ensure permselectivity [29]. Thus, CD SDZ membrane sensors found essential the inclusion of an ionic additive.

3.3. Effect of additive

The role of lipophilic anionic and cationic additives on the potentiometric anion selectivity of the membranes prepared with the PPHR or CD ionophores as anion selective ionophores was examined. The additives were employed to produce ionic sites among the sensing membranes. Generally, this procedure improves the general analytical response of the potentiometric sensor by ensuring that membranes are perm-selective, reducing the ionic interference and lowering the electrical resistance of the membranes [30]. It may also catalyze the exchange kinetics at the sample–membrane interface and enhance the sensitivity of the membrane [22].

In general, with positively charged receptors, lipophilic anionic sites should be added to the membrane in order to optimize the sensor selectivity, whereas in the case of neutral receptors cationic sites should be added [31,32]. In addition, depending on the organic ligand and the metal center, PPHR receptors applied in potentiometric sensors may contain both charged and neutral receptors. The type of ligand and metal center influence the sensor selectivity due to differences in the electron-accepting character of the complex.

Thus, the additives employed for PPHR-based sensors were either positively (TOABr, DDABr) or negatively charged (KTpClPB) while CD-based sensors had always positively charged additives. All these were sufficiently lipophilic to remain solely in the organic membrane phase when in contact with aqueous solution. The effect of carrier:SDZ molar ratios on the sensor response was studied by doping membranes with 25, 50 to 100% additive relative to the ionophore; the corresponding mass ratios are indicated in Table 1, and vary according to the molar mass of each additive.

As a general rule, the positively charged additives enhanced the sensitivity of the PPHR-based sensors from 52 to 60 mV decade^{-1} (Fig. 3B). A slight increase in slope was also observed for an increasing amount of additive. Both limit of detection (LOD) and lower limit of linear range (LLLR) remained unaffected by the additive amount. In turn, it is believed that the anionic additive acted as an excluder of anionic-species (including SDZ), leading to a decreased sensitivity.

The positive additive played a fundamental role in the CD sensors. TOABr increased more significantly the slopes of the CDs with larger cavities while DDABr increased with more relevance the slopes of the smaller carriers. Comparing CD-based sensors with additive, only those with β-CD displayed Nernstian behavior, independently of the amount of additive. The other sensors showed increasing sensitivities for an increased amount of additive.

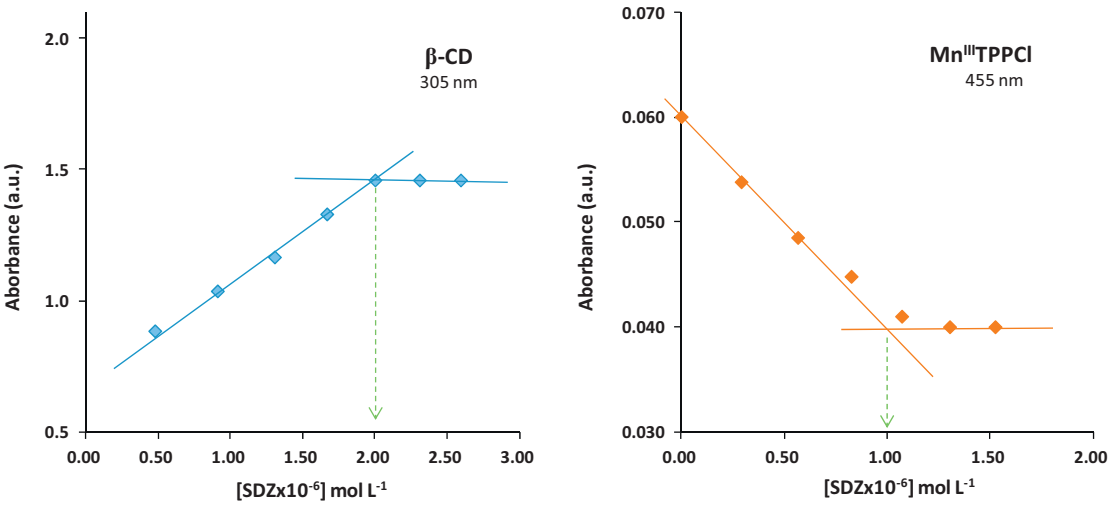

Fig. 2. Molar ratio between SDZ and CD (left) or PPHR (right), for 1.0×10^{-6} mol L^{-1} of ionophore.

Generally, potentiometric sensors with higher amount of additive presented extended working ranges, with lower LLLR and LOD. These results suggest that the cavity size of β-CD was the most suitable one for interacting with the SDZ hydrophobic portion.

3.4. Effect of pH

The effect of pH on the sensor potential was studied for a SDZ solution of 1×10^{-3} mol L^{-1}. The pH was adjusted by small additions of concentrated sulfuric acid or sodium hydroxide solution and recorded by a combined glass-pH electrode. When pH values were plotted against emf (mV), it was observed that the potential was not steady but almost independent from pH in the range of 2–11.0, with potential variations within ±15 mV. Consequently, this interval was considered a plausible pH working range. α-CD sensors with DDABr displayed however a potential drop after pH 7, suggesting an HO^- interference effect. Sensors with Mn^{III}TPPCl/KTpClPB behaved like a pH electrode (Fig. 4), probably in response to its negatively charged additive, present in the same amount as PPHR.

3.5. Response time, lifetime and validation

The time required to achieve a steady potential response (±1 mV) for a 10-fold concentration increase from 5×10^{-5} and 1×10^{-4} mol L^{-1} SDZ was <15 s (Table 1). These results indicated that the complexation process between SDZ and the different ionophores was kinetically fast, pointing out that the overall free energy barrier for the free to complexed states was small enough for complexation to occur rapidly. Replicate calibrations for each sensor indicated low potential drift, long-term stability and negligible change in the response of the sensors.

Having sensors stored and conditioned in 1×10^{-3} mol L^{-1} SDZ solution, the detection limits, response times, linear ranges and calibration slopes were reproducible within ±3% of their original values, and over a period of at least 2 months. This was the maximum period observed, meaning that the sensors could last longer.

During this period, leaching of electroactive materials from the membranes was not perceptible, and ionophore and additive remained preferentially in the hydrophobic phase. PPHR deriva-

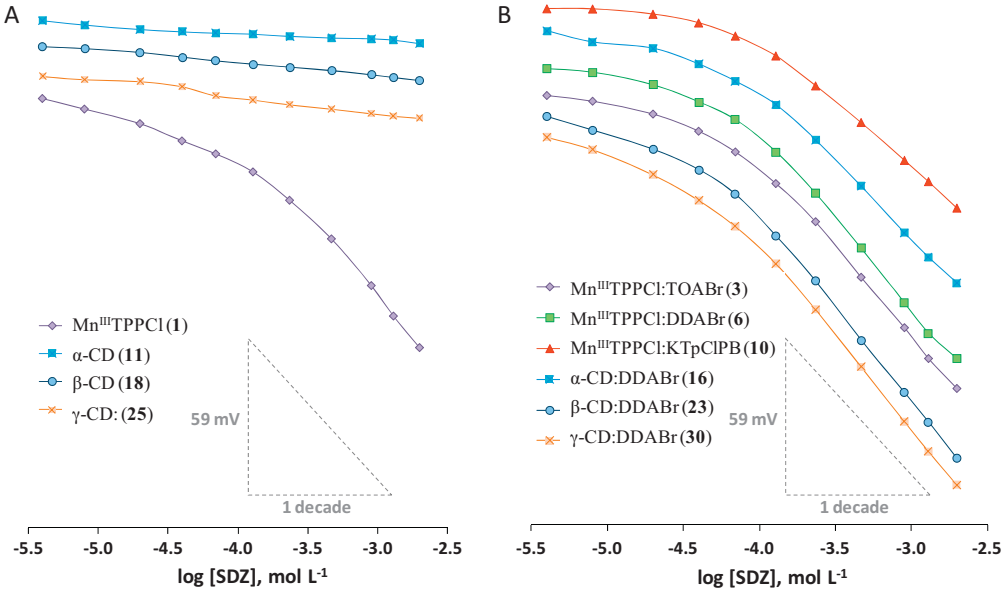

Fig. 3. Typical calibration curves of SDZ sensors without additive (A) or with different additives (B) in HEPES buffer.

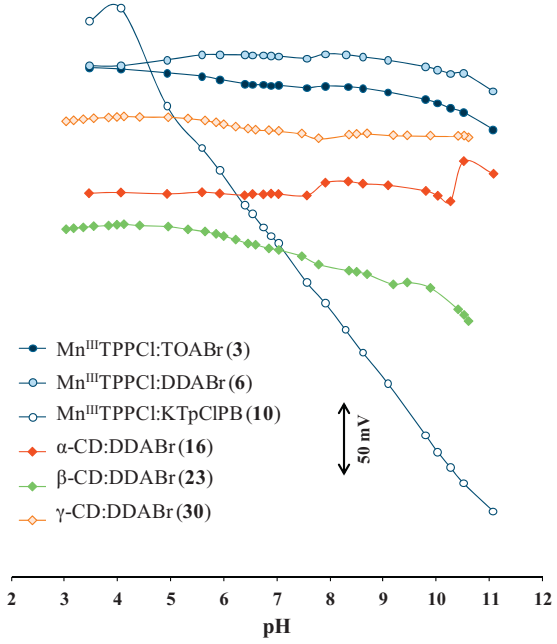

Fig. 4. Influence of pH on the potentiometric response of SDZ sensor ([SDZ] = 1×10^{-3} mol L^{-1}).

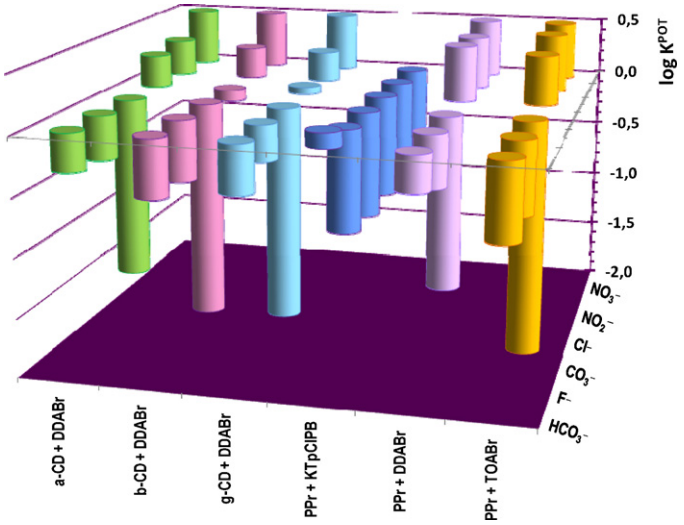

Fig. 5. Potentiometric selectivity coefficients ([SDZ] = 1×10^{-3} mol L^{-1}).

tives are typically hydro-soluble, while CD showed less water solubility (α-Cd, β-Cd and γ-CD are 14.5, 1.85 and 23.2 g, 100 mL^{-1} at 20 °C). Their aromatic rings or uncharged groups are however responsible for allowing them to stand inside a hydrophobic membrane. The water solubility of the ionic additives is much lower than that of the ionophores, because they bear a strong hydrophobic structure.

Validation of the potentiometric data was assessed with regard to precision, accuracy, within-day and between-day variability, and recovery (Table 2). These parameters were selected due to the intended analytical application. In general, all sensors displayed similar and good analytical behavior.

In general, an exceptional potential stability was observed for a solid-contact sensor. This may be attributed to the membrane good adhesion, its impossible lamination by water and its high thickness. The membrane is applied over a cavity where graphite occupies only its deep side [9] and the walls are made of Perspex. Since Perspex dissolves with tetrahydrofuran, the liquid membrane solution reacts with the wall, leading to a dry membrane that constitutes a solid block resistant to water lamination. Hydrated layers are formed therefore by water molecules moving through the plasticized membrane only. Still, the water reaches the graphite because it is responsible for establishing the internal potential, along with O_2 [33,34]. Since the membrane is quite thick (ca. 2 mm), it seems that the water gradient remains constant for a long period of time and the film of water reaching the graphite is unable to drift the potential with significance.

3.6. Sensors selectivity

The selectivity behavior of sensors is defined by the ion exchange constants which depend on the selectivity of complexation as well as on the standard free energies of the respective ions in the aqueous and organic phases [35]. The former requisite suggests the use of ligands that strongly bind the preferred ion and only weakly all the others [35], as the mechanism of selectivity is mainly governed by stereospecific and electrostatic aspects, being the lipophilic environment dictated by the plasticizer. For metalloporphyrins, the kind and oxidation state of the coordinated central are also relevant.

The selectivity of a potentiometric sensor for the main ion (analyte) over other ions present in the solution is usually expressed in terms of the potentiometric selectivity coefficient, $K^{pot}_{A,B}$. The selectivity coefficients were determined by the separated solutions method [36]. The emf values of SDZ or interfering species solutions were measured separately and the corresponding selectivity coefficients were calculated by using the following equation:

$$K^{POT}_{SDZ^-,J^{z-}} = \frac{E_2 - E_1}{S} + \left(1 - \frac{-1}{Z}\right) \times \log C_{SDZ}$$

where E_1 and E_2 are the sensor potentials in 1.0×10^{-3} mol L^{-1} solutions of SDZ and interfering ion J^{Z-}, respectively, C_{SDZ} the concentration of SDZ and S the practical slope of the calibration plot calculated in mV decade^{-1}.

The potentiometric selectivity coefficients of the proposed sensor are shown in Fig. 5. Carbonate, chloride, nitrate, nitrite, fluoride and hydrogencarbonate (bicarbonate) were selected as possible interfering species because they are usually present in drinking water, as well as in wastewaters, industrial effluents, soils, etc.

The presence of cationic sites from DDABr or TOABr into the membrane lead to the following general relative order of interfering effect: nitrate > nitrite > chloride > fluoride ~ hydrogen hydrogencarbonate > carbonate (Table 3). This seems a near-Hofmeister behavior [37]. It is believed that the presence of ionic sites of opposite charge as the analyte ion (as required for neutral carriers), forces uncomplexed ions to be extracted for electroneutrality reasons, which gives a selectivity sequence that reflects the relative lipophilicity of the sample ions and is not influenced by the complexation with the charged ligand [38]. The experimental results also point out that the selectivity behavior did not depend on the ionophore in use, neither Mn^{III}TPPCl nor α-CD, β-CD or γ-CD. The fact of having α, β or γ-CD provided no significant differences between these with regard to selectivity.

Generally, the ionic sites of the same charge sign as the primary ion improved the selectivity. Membranes having PPHR as ionophore and negatively charged additives displayed a completely different selectivity behavior. All possible interfering compounds showed similar potentiometric selectivity coefficients, lying within -0.92 ± 0.06 log K^{POT}. Hydrogen carbonate was the only exception to this observation.

Table 2
Validation data of several SDZ sensors.

Validation	ISEs					
	Mn^{III}TPPCl TOABr (**3**)	Mn^{III}TPPCl DDABr (**6**)	Mn^{III}TPPCl KTpClPB (**10**)	α-CD DDABr (**16**)	β-CD DDABr (**23**)	γ-CD DDABr (**30**)
Cv_w, %	1.99	4.29	2.12	5.67	2.58	1.62
Accuracy, %	1.81	4.83	26.64	0.24	2.21	5.22
Within-day variability, %	1.70	1.91	2.31	1.99	1.32	1.89
Between-day variability, %	2.05	2.13	3.69	2.50	1.64	3.69
Recovery, %	99.1	97.9	98.9	104.3	101.1	98.6

Table 3
Selectivity coefficients for SDZ selective sensors for various interfering ions using separate solution method.

ISEs	$\log K^{POT}_{SDZ,J^{z-}}$					
	CO_3^{2-}	Cl^-	F^-	HCO_3^-	NO_3^-	NO_2^-
Mn^{III}TPPCl + TOABr (3)	−2.04	0.43	−0.63	−0.66	1.39	0.65
Mn^{III}TPPCl + DDABr (6)	−1.49	0.48	−0.45	−0.31	0.99	0.45
Mn^{III}TPPCl + KTpClPB (10)	−0.90	−0.88	−0.86	0.12	−1.02	−0.95
α-CD + DDABr (16)	−1.56	0.28	−0.37	−0.32	1.37	0.31
β-CD + DDABr (23)	−1.86	−0.10	−0.52	−0.50	1.34	0.27
γ-CD + DDABr (30)	−1.84	0.06	−0.30	−0.42	1.48	0.26

3.7. Response mechanism

For PPHRs, the nature of the ionophore anion interaction mechanism correlates with the charge of the metal ion chelated by the PPHR [39,40]. Trivalent metal ion complexes with PPHR were found to serve as either neutral or charged carriers depending on the existence and number of bound axial ligands. In the case of sensors based on neutral carriers, ionic sites with an opposing charge to that of the primary ions are necessary to decrease the membrane resistance, which in turn reduces the co-ion interferences, and achieves a Nernstian response with an acceptable selectivity, and improves the detection limit. In the present study, ion-selective membranes formulated with Mn(III)PPHR required a cationic additive to enhance the potentiometric response. Thus, these results pointed out that the PPHR ionophore acted as neutral carrier in the membrane.

As expected, CDs also acted as neutral carrier. These neutral anion receptors incorporate strong, multiple, hydrogen bond donor groups (–OH) on its surface. Typically, once inside the CD cavity, the guest molecule makes conformational adjustments to take maximum advantage of the weak van der Waals forces that exist [14].

The effect of plasticizer and additive upon the observed potentiometric response was tested by evaluating two news sensors, carrying plasticized membranes (i) with no ionophore and no additive or (ii) with no ionophore and with negatively charged additive. The first sensor was quite unstable and the potential was unable to decrease with increasing SDZ concentrations. Its potential change ranged ±3 mV along each calibration. The second sensor showed sub-Nernstian behavior, with slopes of −25 mV decade^{-1} after a concentration of 1×10^{-4} mol L^{-1} SDZ. Especially for CD-based sensors, this indicated that the ionophore played a significant role on the observed response.

3.8. Complementary sample analysis

The previous selectivity study is mainly theoretical and suggested high interfering effects from nitrate, nitrite and chloride upon the potentiometric response. Thus, before proceeding with samples analysis, the most contaminated waters samples were analyzed in terms of their main chemical composition. The parameters selected for this purpose were those indicated on the Portuguese law for waters. The average levels found were indicated in Table 4. The obtained results are always below the legal limits and suggested that the proposed potentiometric method could be applied to the analysis of waters in aquaculture environment.

3.9. Application

The determination of SDZ in aquaculture waters was carried out on the previously prepared samples by direct potentiometry. The obtained results are summarized in Table 5. The conventionally shaped sensors selected for this purpose were sensors 3 and 23 (see Table 1); they presented increased sensitivity of response (mV decade^{-1}), good potential reproducibility and stability.

The samples were spiked by adding small increments of 1.0×10^{-2} mol L^{-1} standard SDZ solution to 20.0 mL aliquot samples of various concentrations. The change in potential reading was recorded for each increment and used to calculate the concentration of SDZ sample in the sample solutions using the following equation:

$$C_{SDZ} = \frac{C_s \times V_s}{(V_{SDZ} + V_s) \times 10^{\frac{\Delta E}{S}} - V_{SDZ}}$$

Here C_{SDZ} is the SDZ concentration of testing sample, C_s is the concentration of the standard, V_x and V_s are the corresponding volumes, S is the slope of the potentiometric response, and ΔE is the change in potential [41].

Table 4
Chemical parameters of the water samples.

	Sample A	Sample B	Sample C
Turbidity (UNT)	<0.2	<0.2	0.7
pH (Sorensen scale)	7	6.9	6.7
Conductivity (μS cm^{-1})	41	41	135
Nitrate (mg L^{-1})	1.7	1.3	12.1
Nitrite (mg L^{-1})	<0.03	<0.03	<0.03
Ammonium (mg L^{-1})	<0.05	0.07	<0.05
TOC (mg L^{-1})	<1	<1	<1
Cu (mg L^{-1})	<0.01	<0.01	<0.01
BOD (mg L^{-1})	<3	<3	<3
COD (mg L^{-1})	<30	<30	<30

TOC: total organic content; BOD: biochemical oxygen demand; COD: chemical oxygen demand.

Table 5
Determination of SDZ in aquaculture by applying the standard addition method and the corresponding statistical data.

Sample	Added, mol L^{-1}	Found, mol L^{-1} ($n=3$)	RD (%) ($n=3$)	Recovery(%) ($n=3$)
1	2.55×10^{-4}	$2.45\times10^{-4}\pm1.2\times10^{-5}$	4.0	96.0
	5.39×10^{-4}	$5.22\times10^{-4}\pm2.6\times10^{-5}$	3.0	97.0
	1.06×10^{-3}	$1.03\times10^{-3}\pm4.6\times10^{-5}$	3.0	97.0
2	2.55×10^{-4}	$2.62\times10^{-4}\pm3.2\times10^{-5}$	−2.9	102.9
	5.39×10^{-4}	$5.51\times10^{-4}\pm5.6\times10^{-5}$	−2.3	102.3
	1.06×10^{-3}	$1.01\times10^{-3}\pm7.2\times10^{-5}$	4.4	95.6
3	2.55×10^{-4}	$2.39\times10^{-4}\pm1.1\times10^{-5}$	6.4	93.6
	5.39×10^{-4}	$5.30\times10^{-4}\pm3.0\times10^{-5}$	1.6	98.4
	1.06×10^{-3}	$1.07\times10^{-3}\pm2.1\times10^{-5}$	−1.1	101.1
4	2.55×10^{-4}	$2.54\times10^{-4}\pm2.2\times10^{-5}$	0.5	99.5
	5.39×10^{-4}	$5.54\times10^{-4}\pm5.0\times10^{-5}$	−2.9	102.9
	1.06×10^{-3}	$1.03\times10^{-4}\pm6.9\times10^{-5}$	3.0	97.0
5	2.55×10^{-4}	$2.49\times10^{-4}\pm1.1\times10^{-5}$	2.2	97.8
	5.39×10^{-4}	$5.07\times10^{-4}\pm1.5\times10^{-5}$	5.8	94.2
	1.06×10^{-3}	$1.05\times10^{-3}\pm1.7\times10^{-5}$	1.2	98.8
6	1.07×10^{-4}	$1.01\times10^{-4}\pm1.7\times10^{-5}$	5.5	94.5
	5.39×10^{-4}	$5.19\times10^{-4}\pm2.4\times10^{-5}$	3.6	96.4
	1.06×10^{-3}	$1.00\times10^{-3}\pm3.7\times10^{-5}$	5.4	94.6

Found: mean ± standard error; RD: relative deviation.

The values reported in Table 5 were calculated from three determinations in aquaculture water samples collected from different farming places. The obtained results confirmed the accuracy and precision of the present work. Recoveries ranged 93.6 and 102.9%, thus confirming the accuracy of the analytical results. Relative standard deviations were also low, and confirmed the precision of the proposed method. Student *t* test (at 95% confidence level) confirmed the accuracy of the analytical data because

Table 6
The main features of the proposed method and previously reported sensors for SDZ determination.

Method	Small description	Samples	Detection limit	Response time	Reference
Fluorimetry	Competitive immunocomplex capture format making use of an immobilized protein A/G sorbent or a restricted access support in a novel homogeneous–heterogeneous (HH) assay mode.	Water Honey	0.11 μg L^{-1} 0.85 μg L^{-1}	18 min 2 min	[42]
SPR	Immunoassays based on the plasmon of gold diffraction grating surface for simultaneous detection of antibiotics from different groups, including sulfapyridine as a sulphonamide compound.	Milk	0.29 μg L^{-1}	≈30 min	[43]
SPR	Inhibition assay format in an optical biosensor chip.	Chicken serum	7–1000 μg L^{-1}	≈10 min	[47]
WIOS	Lab-on-a-chip for multi-antibiotic competitive immunoassay based on competitive immunoassay based on wavelength interrogated optical sensor technology and a polymer-based self-contained microfluidic cartridge.	Milk	100 μg L^{-1}	10 min	[44]
WIOS	Competitive immunoassay format using immunoreagents previously developed for the generic detection of sulfapyridine and evaluated by enzyme-linked immunosorbent assay. The immunoreagents were immobilized onto the surface of the waveguide chip, and a fluidic cell allowed flowing analyte and detection solutions above the surface.	Milk	0.5 μg L^{-1}	≈30 min	[45]
Electrochemistry	The immunological reaction for the detection of sulfonamide antibiotics performed on the magnetic bead is based on a direct Competitive assay using a tracer with horserodish peroxidase for the enzymatic labeling and modified magnetic beads captured by a magneto sensor made of graphite–epoxy composite acting as the transducer.	Cream milk	1.44 μg L^{-1}	–	[46]
Potentiometry	PVC membrane selective electrodes for SDZ for flow and batch measurements with iron phthalocyanine as ionophores	Drugs Biol. fluids	0.87–7.0 mg L^{-1}	<30 s	[9]
Potentiometry	PVC membranes with Mn^{III}TPPCl, α-CD, β-CD, γ-CD molecules as ionophores.	Aquaculture Water	3 mg L^{-1}	<30 s	This work

UV/Vis: Ultraviolet Visible; SPR: Surface Plasmon Resonance; WIOS: sensitive wavelength interrogated optical sensor.

the calculated t (0.28) did not exceed the theoretical value (2.07).

In order to know if the proposed method exhibited any fixed or proportional bias, a simple linear regression of the taken amounts against found was calculated. A small displacement of the zero origin (equal to 1×10^{-5}) and of the unit slope (0.9736) was found, confirming the absence of the above bias.

3.10. Comparison to previous sensors

The proposed work is compared in terms of analytical figures of merit with other sensors previously reported in the literature for the determination of different sulphonamides (Table 6). In general terms, it is possible to conclude that the main advantage of the presented work is the low response time and the low cost the proposed sensors. They display very quick responses and are inexpensive in terms of regular laboratory materials. Their main disadvantage compared to others is the high limit of detection. This last feature is mostly correlated to the use of conventional materials instead of nanostructured ones, which are not included in elegant platforms. Thus, further work is conducted to incorporate the membranes upon microfluific devices. Of course, this will turn out more expensive, but much cheaper than all other reported methods. These rely only on immunoassays and therefore require highly expensive consumable reagents. Furthermore, some of these use very expensive transducers that are quite far from the regular potentiometer in everyday laboratory.

The proposed sensors offer similar analytical features to those in [9]. The here presented sensor offers however higher stability in terms of analytical signal. The use of regular CD compounds as sensors is also an advantage in terms of routine laboratory.

4. Conclusions

Mn(III) PPHR and CD were suitable ionophores for the preparation of SDZ sensors. They acted both as neutral carriers. The presence of positively charged additives in the selective membrane enhanced the potentiometric performance. Selectivity profiles followed a near-Hofmeister pattern with the exception of PPHR-based sensors with negatively charged additives. Despite their good selectivity behavior, they suffered from a great pH interference effect.

The proposed sensors were found useful for the control of SDZ in waters from aquaculture origin. The corresponding detectors were constructed in a simple and inexpensive way. The overall procedure was considered precise, accurate, and inexpensive regarding reagent consumption and equipment involved. Considering its routine application, the main advantages arise from the composition and quantity of emitted effluents, with small concern in terms of environmental issues. The proposed method also enabled high sampling frequencies with low operator intervention, meaning that it was suitable for the routine procedures carried out in analytical laboratories; it is particularly suitable for screening assays.

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References

[1] S.A.E. Kools, J.F. Moltmann, T. Knacker, Regul. Toxicol. Pharmacol. 50 (2008) 59–65.
[2] H.C. Wegener, F.M. Aarestrup, P. Gerner-Smidt, F. Bager, Acta Vet. Scand. Suppl. 92 (1999) 51–57.
[3] I. Braschi, S. Blasioli, L. Gigli, C.E. Gessa, A. Alberti, A. Martucci, J. Hazard. Mater. 178 (2010) 218–225.
[4] E. Zuccato, S. Castiglioni, R. Bagnati, M. Melis, R. Fanelli, J. Hazard. Mater. 179 (2010) 1042–1048.
[5] Z. Zhang, V.V. Cosofret, Select Elect. Rev. 12 (1990) 35–135.
[6] V.V. Cosofret, R.P. Buck, Pharmaceutical Applications of Membrane Sensors, CRC Press, Boca Raton, FL, 1992, p. 448.
[7] A.R. Fakhari, M. Alagheman, M. Shamsipur, Anal. Lett. 34 (2001) 1097–1106.
[8] V.V. Cosofret, R.P. Buck, Crit. Rev. Anal. Chem. 24 (1993) 1–58.
[9] A.H. Kamel, F.T.C. Moreira, S.A.A. Almeida, M.G.F. Sales, Anal. Sci. 25 (2009) 365–371.
[10] S. Amemiya, Potentiometric ion-selective electrodes, in: C.G. Zoski (Ed.), Handbook of Electrochemistry, Elsevier, 2007 (Chapter 7).
[11] M.R. Ganjali, P. Norouzi, M. Rezapour, Encyclopedia of Sensors, Potentiometric Ion Sensors, American Scientific Publishers, Los Angeles, vol. 8, 2006, 197–288.
[12] M.R. Ganjali, P. Norouzi, M. Rezapour, F. Faridbod, M.R. Pourjavid, Sensors 6 (2006) 1018–1086.
[13] J.W. Steed, D.R. Turner, K.J. Wallace, Core Concepts in Supramolecular Chemistry and Nanochemistry, University of Southern Mississippi, John Wiley & Sons, USA, England, 2007.
[14] E.M.M. Del Valle, Proc. Biochem. 39 (2004) 1033–1046.
[15] M. Biesaga, K. Pyrzynska, M. Trojanowicz, Talanta 51 (2000) 209–224.
[16] E.D. Steinle, U. Schaller, M.E. Meyerhoff, Anal. Sci. 17 (1998) 79–84.
[17] J. Yoon, J.H. Shin, I.R. Paeng, H. Nam, G.S. Cha, K.J. Paeng, Anal. Chim. Acta 367 (1998) 175–181.
[18] M.M.G. Antonisse, B.H.M. Smellink-Rubel, J.F.J. Engbersen, D.N. Reinhoudt, J. Chem. Soc., Perkin Trans. 2 (1998) 773–778.
[19] Z. Deyl, I. Miksik, A. Eckhardt, V. Kasicka, V. Král, Curr. Anal. Chem. 1 (2005) 103–119.
[20] Y.A. Zolotov, Macrocyclic Compounds in Analytical Chemistry, John Wiley and Sons Ltd, New York, 1997, p. 448.
[21] R.P. Buck, V.V. Cosofret, Pure Appl. Chem. 65 (1993) 1849–1858.
[22] E.M.G. Santos, A.N. Araújo, C.M.C.M. Couto, M.C.B.S.M. Montenegro, J. Pharm. Biomed. Anal. 42 (2006) 535–542.
[23] E. Bakker, P. Buhlmann, E. Pretsch, Chem. Rev. 97 (1997) 3083–3132.
[24] Y. Qin, E. Bakker, Anal. Chem. 76 (2004) 4379–4386.
[25] I. Beletskaya, V.S. Tyurin, A. Yu, A. Tsivadze, R. Guilard, C. Stern, Chem. Rev. 109 (2009) 1659–1713.
[26] W. Zhang, E. Rozniecka, E. Malinowska, P. Parzuchowski, M.E. Meyerhoff, Anal. Chem. 74 (2002) 4548–4557.
[27] T. Akimoto, Yakugaku Zasshi 125 (2005) 971–980.
[28] G. Huschek, D. Hollmann, N. Kurowski, M. Kaupenjohann, H. Vereecken, Chemosphere 72 (2008) 1448–1454.
[29] E. Bakker, E. Pretsch, Trends Anal. Chem. 21 (2001) 11–19.
[30] M. Telting-Diaz, E. Bakker, Anal. Chem. 73 (2001) 5582–5589.
[31] D. Ammann, W.E. Morf, P. Anker, P.C. Meier, E. Pretsch, W. Simon, Ion-Sel. Electrode Rev. 5 (1983) 3–92.
[32] P.M. Gehring, W.E. Morf, M. Welti, E. Pretsch, W. Simon, Helv. Chim. Acta 73 (1990) 203–212.
[33] A. Hulanicki, E.M Trojanowicz, Anal. Chim. Acta 87 (1976) 411–417.
[34] G. Herdecke, J. Kropf, G. Stork, J.G. Schindler, Z. Fresenius, Anal. Chem. 303 (1980) 364–370.
[35] E. Bakker, E. Pretsch, Angew. Chem. Int. Ed. 46 (2007) 5660–5668.
[36] Y. Umezawa, K. Umezawa, H. Sato, Pure Appl. Chem. 67 (1995) 507–518.
[37] E. Malinowska, E. Niedziółka, M.E. Meyerhoff, Anal. Chim. Acta 432 (2001) 67–78.
[38] M.M.G. Antonisse, D.N. Reinhoudt, Electroanalysis 11 (1999) 1035–1048.
[39] H. Jafar, M.K. Amini, H. Motaghi, S. Tangestaninejad, M. Moghadam, Sens. Actuat. B 87 (2002) 448–456.
[40] E. Bakker, E. Malinowska, R.D. Schiller, M.E. Meyerhoff, Talanta 41 (1994) 881–890.
[41] R.P. Buck, E. Lindner, Pure Appl. Chem. 66 (1994) 2527–2536.
[42] D. Jorneta, M.A. González-Martíneza, R. Puchades, A. Maquieira, Talanta 81 (2010) 1585–1592.
[43] F. Fernández, K. Hegnerová, M. Piliarik, F. Sanchez-Baeza, J. Homola, M.P. Marco, Biosens. Bioelectron. 26 (2010) 1231–1238.
[44] G. Suárez, Y.H. Jin, J. Auerswald, S. Berchtold, H.F. Knapp, J.M. Diserens, Y. Leterrier, E.M. Jan-Anders, G. Voirin, Lab Chip 9 (2009) 1625–1630.
[45] J. Adrian, S. Pasche, J.M. Diserens, F. Sánchez-Baeza, H. Gao, M.P. Marco, G. Voirin, Biosens. Bioelectron. 24 (2009) 3340–3346.
[46] E. Zacco, J. Adrian, R. Galve, M.P. Marco, S. Alegret, M.I. Pividori, Biosens. Bioelectron. 22 (2007) 2184–2191.
[47] M. Bienenmann-Ploum, T. Korpimaki, W. Haasnoot, F. Kohen, Anal. Chim. Acta 529 (2005) 115–122.