New and low cost plastic membrane electrode with low detection limits for sulfadimethoxine determination in aquaculture waters

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ABSTRACT

Sulfadimethoxine (SDM) is one of the drugs, often used in the aquaculture sector to prevent the spread of disease in freshwater fish aquaculture. Its spread through the soil and surface water can contribute to an increase in bacterial resistance. It is therefore important to control this product in the environment. This work proposes a simple and low-cost potentiometric device to monitor the levels of SDM in aquaculture waters, thus avoiding its unnecessary release throughout the environment. The device combines a micro- pipette tip with a PVC membrane selective to SDM, prepared from an appropriate cocktail, and an inner reference solution. The membrane includes 1% of a porphyrin derivative acting as ionophore and a small amount of a lipophilic cationic additive (corresponding to 0.2% in molar ratio). The composition of the inner solution was optimized with regard to the kind and/or concentration of primary ion, chelating agent and/or a specific interfering charged species, in different concentration ranges. Electrodes con- structed with inner reference solutions of 1×10^{-8} mol/L SDM and 1×10^{-4} mol/L chromate ion showed the best analytical features. Near-Nernstian response was obtained with slopes of −54.1 mV/decade, an extraordinary detection limit of 7.5 ng/mL (2.4×10^{-8} mol/L) when compared with other electrodes of the same type. The reproducibility, stability and response time are good and even better than those obtained by liquid contact ISEs.

Recovery values of 98.9% were obtained from the analysis of aquaculture water samples.

Keywords:
Lowering limit of detection, Ion-selective electrodes, Inner reference solution, Liquid-contact electrodes, Electrode body of micropipette tips

1. Introduction

Ion-selective electrodes (ISEs) have been widely known for their ability to selectively determine a wide variety of charged analytes [1]. Traditionally, an ISE is a sensor where an ion-selective membrane separates two solutions: the sample solution where the concentration of analyte is unknown and a known internal reference solution of fixed analyte concentration [2,3]. This classical arrangement (used with primary ions that have relatively high activities in the internal solution) results in sensors of stable and reproducible standard potentials, usually with linear responses of about 10^{-6} mol/L.

ISEs of lower detection limits ('''10^{-8} mol/L) are obtained when the composition of the inner reference solution is suitably chosen [4]. In essence, the electrical potential which ISEs produce is generated from ion-transfer processes, across the interface between the sample and membrane solution [5]. Conventionally, when the concentration of primary ions in the sample solution is very low and there are high levels of primary ions leaching from the membrane, the diffusion across the membrane is high, implying that high limits of detection (LODs) will be achieved. One way to prevent this conventional ion flux is to force a flux of primary ions in the opposite direction, i.e., towards the inner solution [6].

With the aim of achieving this purpose, numerous parameters must be carefully selected. This includes the composition of the inner electrolyte and the selective membrane [7]. After ensuring the optimum selectivity properties of the PVC membrane, it is important to evaluate the effect of several parameters of the inner reference solution, including the primary ion concentration and the kind/amount of suitable complexing agents, capable of extracting the primary ion from the selective membrane [5]. In this work, both the selective membrane and inner electrolyte composition were checked in order to attain even lower LODs. Following previous studies, this work was applied to detect/quantify a sulphonamide antibiotic in environmental waters at levels lower than those previously detected by potentiometric devices.

Sulphonamides have a wide spectrum of action against most Gram+ and many Gram− microorganisms. These drugs are commonly used in aquaculture to prevent/treat fish diseases. However,

due to their high water solubility [8], they end up being released throughout the environment and find their way into soils, sediments and groundwater [9]. This practice has been correlated to the appearance of resistant bacteria and has given rise to significant public concern. Since these antibiotics are among the range of drugs used in human therapy, the infection of humans by such resistant bacteria would in turn pose a serious public health threat.

SDM (Fig. 1) is one of these sulphonamide antibiotics used in freshwater aquaculture. It has been routinely determined by conventional optical and electrical methods [10,11], HPLC-based procedures [12] and ELISA [13] but a single method that could lead to a low cost procedure with limits of detection capable of on-site application in aquaculture waters has never been envisaged.

In the present work, potentiometric sensors are described for SDM with the objective of reaching very low LODs by carefully selecting the selective membrane and inner reference compositions. These devices use a micropipette tip as electrode body, thereby constituting a very low cost alternative for practical application.

2. Experimental

2.1. Reagents and solutions

All chemicals were of analytical grade and de-ionized water (conductivity < 0.1 μS/cm) was employed. SDM, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), tetraoctylammonium bromide (TOABr) and *meso*-tetraphenylporphyrin manganese (III) chloride complex (Mn^{III}TPPCl) were purchased from Sigma–Aldrich. Poly(vinyl chloride) (PVC) of high molecular weight and *o*-Nitrophenyloctyl ether (*o*NPOE) were Fluka and tetrahydrofuran (THF) was Riedel-deHäen.

Stock solutions of 1.0×10^{-4} mol/L SDM were prepared in water. Less concentrated SDM standards were prepared by accurate dilution of the previous solution in buffer. Buffer solutions consisted of 1.0×10^{-4} mol/L HEPES.

The extent of interference from some species such as carbonate, chlorate, chloride, chromate, cyanide, fluoride, hydrogenocarbonate, nitrate, nitrite, persulphate, phosphate, salicylate and sulphate solution was evaluated. For this purpose, solutions of the sodium salts of these compounds (prepared in Hepes buffer) were used. Several solutions containing SDM, beta-cyclodextrin (b-CD) as quelating agent and chromate as interferent in different concentrations were prepared and used as internal filling solution for the assembled electrodes. Membranes were conditioned in 1×10^{-8} mol/L SDM solution overnight, before measurements were taken. The low concentration of this solution aimed to minimize the effect of co-extraction from the sample side during conditioning.

2.2. Apparatus

All potentiometric measurements were performed at room temperature (21 °C). Emf measurements were conducted in stirred solutions using a Crison GLP 21 pH meter stir plate and taken against an Ag/AgCl reference electrode, prepared by dipping the silver wire in 1×10^{-3} mol/L iron (III) chloride solution. The potentiometric cell assembly was as follows: micropipette tip |SDM selective membrane| buffered sample solution (1×10^{-4} mol/L HEPES)|Ag/AgCl reference. Fig. 2A shows the different steps involved in constructing the electrodes (Fig 2A) and the reading system comprised of the switch and potentiometer (Fig 2B). Each way presented an electrical antenna connector which provided suitable adaptation to each electrode. Spectrophotometric assays when necessary were carried out on a Shimadzu Pharmaspec UV-1700.

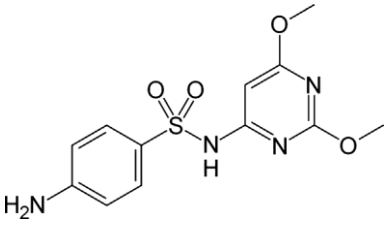

Fig. 1. Chemical structure of SDM.

2.3. Preparation and construction of the SDM sensor

The selective membranes were prepared with different compositions ranging from 2.5to 8.5 mg of Mn^{III}TPPCl (acting as ionophore), 136 to 270 mg from PVC (as polymeric support, previously dissolved in about 4 ml THF), 136 to 270 mg of *o*-NPOE (as plasticizer) and 1.1 mg of TOABr (as additive), according to the data presented in Table 1.

Each resulting homogenous mixture was cast over graphite-based conductive supports, deposited on the edge of a Perspex cylinder tube and to which an electrical wire had been connected, as described elsewhere [14]. ISEs made with micropipette tips confer the best membrane composition when applied at the end of the tip. The membrane was applied by immersing the tip in the membrane solution for a few seconds before being removed and let dry overnight at room temperature. A 0.02–0.15 mm green membrane was obtained, conditioned in a 1.0×10^{-8} mol/L SDM aqueous solution before use and when not in use. An Ag wire covered by AgCl acted as internal reference electrode inside the pipette tip (Fig. 2).

2.4. Potentiometric procedures

All potentiometric measurements were carried out at room temperature and under constant stirring. Increasing concentrations of SDM were obtained by transferring 0.020–2.5 mL aliquots of 1.0×10^{-4} mol/L SDM aqueous solution to a 100 mL beaker containing 40 mL of 1.0×10^{-4} mol/L of suitable buffer. The potential readings of the stirred SDM solutions were measured at room temperature after stabilization at ±0.2 mV and plotted as a function of logarithm SDM concentration.

Selectivity studies were performed by the Matched Potential Method (MPM). The initial concentration of SDM was set at 1×10^{-5} mol/L and the potential decreased ‴15 mV after adding 5×10^{-6} mol/L SDM to this initial concentration. Solutions of interfering species were then added to a fresh SDM solution of 1×10^{-5} mol/L, until the same potential change was observed.

2.5. Analytical application

The analytical usefulness of the developed electrodes was demonstrated by determining SDM in aquaculture water samples collected in the north of Portugal over the summer season. A composite aquaculture water sample was collected from about 2 to 6 vertical profiles and split into appropriate containers at each site.

3. Results and discussion

3.1. Ionophore binding to sulfadimethoxine

Before moving to ISE performance, binding assays between SDM and Mn^{III}TPPCl were carried out to confirm that this was a suitable ionophore for SDM and which would allow ISEs with good selectivity features to be prepared. This binding study was

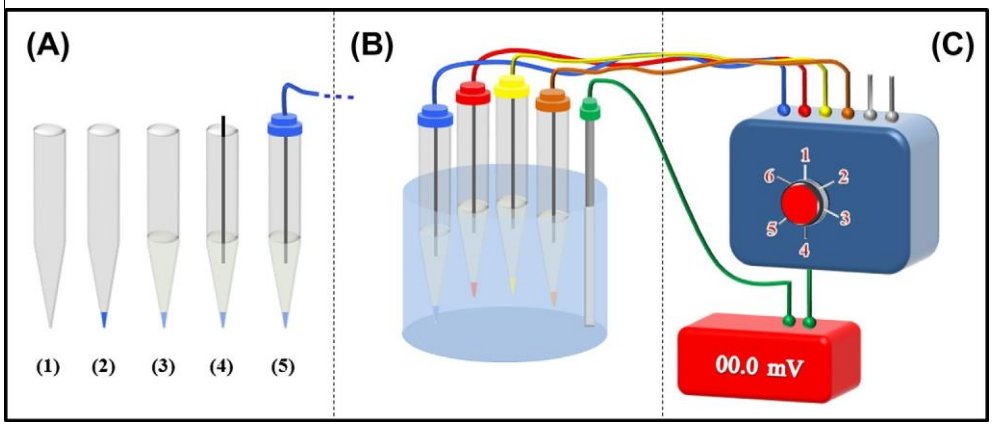

Fig. 2. Schematic diagram of (A) the several stages of the construction of SDM electrodes (1: empty 1000 L pipette tip; 2: application of the selective membrane on the top-end; 3: filling with inner solution; 4: cupper wire adaptation; 5: electrical contact connection), (B) the potentiometric cell and (C) the multi commutation point connected to the potentiometer.

Table 1
Analytical features for SDM electrodes prepared with different selective membrane composition.

Characteristics	Ionophore (mg)			PVC (mg)			Membrane thickness (mm)		
	2.5	5.5	8.5	136	180	270	0.2	0.6	1.5
Additive, 0.2% (wt%)[a]	TOABr	TOABr	TOABr	TOABr	TOABr	TOABr	TOABr	TOABr	TOABr
Plasticizer	oNPOE	oNPOE	oNPOE	oNPOE	oNPOE	oNPOE	oNPOE	oNPOE	oNPOE
Slope, mV/decade	−49.0	−50.7	−43.2	−46.7	−48.9	−47.3	−37.3	−48.5	−48.3
	(±1.13)	(±0.700)	(±2.03)	(±2.11)	(±1.05)	(±1.42)	(±0.0685)	(±0.117)	(±0.559)
R^2 ($n = 3$)	0.998	0.997	0.995	0.993	0.995	0.995	0.997	0.996	0.994
LLLR, ng/mL	83.1	33.2	124	64.8	33.2	24.9	124	83.0	124
LOD, ng/mL	25.6	10.1	37.7	19.6	10.1	7.54	38.8	25.2	37.7
Cv_w, %	2.30	1.38	4.69	4.51	2.15	3.00	0.18	0.241	1.22
Within-day variability, %	1.91	2.40	3.04	1.57	1.32	1.72	4.03	0.849	0.919
Between-day variability, %	1.46	1.72	2.25	1.76	1.46	1.83	3.04	0.630	0.653
Recovery, %	95.1	98.7	92.2	93.2	97.9	95.5	97.1	98.8	98.1

[a] Molar ratio to ionophore.

conducted by recording UV/Vis spectra from 200 to 550 nm. Two of the peaks observed were of major relevance: the peak at 260 nm was due to the presence of SDM/porphyrin complex while the peak at 470 nm was correlated to the presence of free Mn^{III}TPPCl (coloured). These maximum wavelengths were identified by plotting the spectra of a solution with individual and combined solutions of porphyrin and analyte with 1.0×10^{-5} mol/L. Fig. 3 shows the absorption spectra of Mn^{III}TPPCl (1×10^{-5} mol/L) containing various concentrations of SDM. As the concentration of SDM increased,

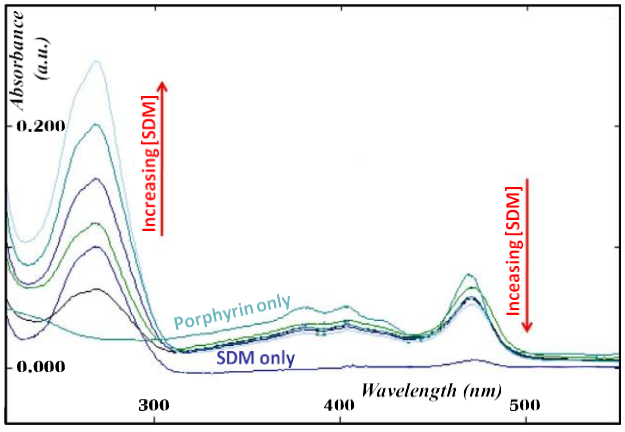

Fig. 3. Absorbance spectra of MnIIITPPCl (1×10^{-5} mol/L) in buffer HEPES 1×10^{-4} mol/L containing various concentration of SDM.

the absorbance at 260 nm increased while the absorbance at 470 nm decreased, thus confirming the complex formation between Mn^{III}TPPCl and SDM.

The molar ratio between the analyte and ligand was calculated by adding 300 1L aliquots of a more concentrated SDM solution to a suitable volume of 1.0×10^{-5} mol/L porphyrin solution. The spectrophotometric spectra of all these was followed for 24 h, recording the individual spectra every 4 h. Overall, the complex formation was immediate (<30 s) and stable over the 24 h. A 1:1 stoichiometry was observed, with a double reciprocal plot exhibiting a linear relationship fitting Eq. (1) [15,16]:

$$1/(A - A_0) = 1/a + 1/aK_1[Mn^{III}TPPCl - SDM]_0 \quad (1)$$

In this equation A, A_0, a, K_1 and $[Mn^{III}TPPCl\text{-}SDM]_0$ are the absorbance of Mn^{III}TPPCl in the presence of SDM, the absorbance of Mn^{III}TPPCl in the absence of SDM, a constant value, the equilibrium constant for the formation of 1:1 Mn^{III}TPPCl-SDM inclusion complex and initial concentration of SDM, respectively. The value obtained equilibrium constant was 1.6×10^7 L/mol, thus confirming a high affinity between SDM and Mn^{III}TPPCl.

3.2. Preliminary studies in solid-contact electrodes

Considering that sensitivity and selectivity of a potentiometric selective membrane depends greatly on its components [17–19], a preliminary membrane composition was estimated using PVC as the polymeric support [20]. The plastic membrane prepared was applied in a solid contact electrode. Since SDM is an antibiotic

from the sulphonamide group, our previous studies had indicated that Mn^{III}TPPCl is a suitable ionophore for ion sensing with potentiometric transduction [21] performing well with an *o*-NPOE plasticizer. In addition, this plasticizer is physically compatible with the polymer, attributing a homogenous character to the membrane solution.

Therefore, the first membrane composition was set at 1 wt.% Mn^{III}TPPCl (5.5 mg), 66 wt.% *o*-NPOE (360 mg) and 33 wt.% PVC (180 mg), employing the conventional relative amounts of all ingredients. The resulting devices exhibited a linear correlation of emf against log[SDM, mol/L] from 3.0×10^{-5} to 2.0×10^{-3} mol/L (9.96–664 μg/mL), with average slopes of -52.3 ± 2.07 mV/decade and a detection limit of 3.02 μg/mL.

To improve the analytical performance, some other membranes were prepared, this time including a charged lipophilic additive. This type of additive is expected to diminish the electrical resistance of the membrane and its control permeselectivity. A 0.2% molar ratio to ionophore was selected for this purpose, ensuring that the performance of the electrode would still be governed by the porphyrin-based ionophore. The cationic additive employed was TOABr, also following our previous studies with sulphonamide antibiotics [22]. The major improvement observed was a shift to a Nernstian behaviour, with a slope of −57.0 ± 1.63 mV/decade. Consequently, further membranes were always prepared with additive, setting its amount at 0.2% of ionophore (in molar ratio).

3.3. *Effect of the selective membrane composition*

Optimum performance of liquid-contact electrodes requires a carefully selected membrane composition. This study started by optimizing the amount of ionophore (Table 1) by considering it as a core ingredient. Different membranes were prepared including 2.5, 5.5 or 8.5 mg of ionophore, along with 360 mg of *o*-NPOE, 180 mg PVC and 0.2% of additive, expressed in molar quantity. The corresponding % of ionophore in the selective membrane was 0.5, 1.0 or 1.5% (in mol). Overall, electrodes with lower amounts of ionophore showed similar near-Nernstian responses (‴50 mV/decade) but those with 1.0% ionophore displayed lower limits of detection (10.1 ng/mL) across a wider dynamic linear concentration range.

The PVC/plasticizer ratio was then studied. This ratio is expected to change the diffusion coefficient of the membrane [23–24] and thus the overall potentiometric performance. The amount of PVC introduced in the membrane was 136, 180 or 270 mg (Table 1). These membranes were set with a constant amount of *o*-NPOE, equal to 540 mg. Overall, the greater the amount of PVC, the better the analytical performance. All other membranes showed similar sensitivities, for different dynamic concentration linear ranges. Membranes with 42% PVC presented the best Lower Limit of Linear Range (LLLR) and Limit of detection (LOD) parameters. However, this was coupled to unstable emf values and longer response times. Thus, the overall best compromise between LOD and LLLR was found for a 33% PVC and this was selected for further studies.

The effect of membrane thickness was also tested for 0.20, 0.60 or 1.5 mm membranes, measured after drying and before conditioning. Typically, membranes of greater thickness are expected to show increased resistance and longer response time but this also depends on the osmotic pressure exerted by the inner reference solution. In this study, the slope became smaller for very thin membranes (−37.3 mV/decade, Table 1). Comparing membranes with 0.6 or 1.5 mm, the former showed lower LLLR and LOD. A thickness of 0.6 mm was therefore selected in further studies.

In short, the composition of the selective membrane was set at 5.5 mg ionophore with a 0.2% molar amount of additive, 33% PVC and 66% plasticizer, with an overall thickness of 0.6 mm.

3.4. *Overall composition of inner reference solution*

Managing the composition of the inner reference solution provides a means to reach very low LODs in potentiometric sensing. This includes the main ion-concentration and the existence of some complexing agent or interfering species that may affect the primary ion. These variables were tested and the results presented in Table 2.

To force the primary ion-flux across the selective membrane towards the inner compartment, a low concentration of this ion should be set at this side of the membrane [4]. However, very low concentrations may promote a high flux, associated with a super-Nernstian response at intermediate concentrations and relative insensitivity at very low analyte levels [25]. In the present study, the concentrations of SDM were set at 1×10^{-7}, 1×10^{-8} and 1×10^{-9} mol/L (Table 2, ISES I, II and III). Although little differences were observed within these SDM concentrations, the concentration of 1×10^{-8} mol/L led to a slightly higher sensitivity, lower LOD and good emf stability. The lower SDM concentration tested generated a greater instability in emf response, suggesting that lower concentrations would not be advisable.

An additional attempt to direct the SDM flux in the membrane towards the inner solution was made by adding a ligand to this solution which was expected to bind SDM. This ligand would help to extract the primary ion from the membrane at the membrane/inner reference solution interface. It has been shown that b-CD

Table 2
Analytical features for SDM electrodes prepared with internal solutions of different composition.

Characteristics	SDM (mol/L)			SDM + b-CD (mol/L)			SDM + Interferents (mol/L)				SDM + Chromate (mol/L)			
	I 1×10^{-7}	II 1×10^{-8}	III 1×10^{-9}	IV 1×10^{-2}	V 1×10^{-4}	VI 1×10^{-6}	VII Chromate	VIII Persulphate	IX Salicylate	X Chlorate	XI 1×10^{-3}	XII 1×10^{-4}	XIII 1×10^{-5}	XIV 1×10^{-6}
Slope, mV/decade	−47.0 (±0.203)	−50.0 (±0.752)	−47.2 (±1.60)	−51.0 (±0.991)	−52.8 (±1.56)	−48.7 (±0.549)	−53.4 (±0.350)	−52.0 (±1.94)	−55.8 (±0.520)	−49.5 (±1.89)	−52.9 (±0.512)	−54.1 (±0.196)	−51.8 (±0.392)	−49.9 (±1.26)
R^2 ($n = 3$)	0.991	0.993	0.990	0.997	0.994	0.992	0.994	0.996	0.995	0.997	0.996	0.998	0.999	0.994
LLLR, ng/mL	64.8	64.8	64.8	64.8	64.8	64.8	24.9	58.2	83.1	83.1	64.8	24.9	36.3	64.8
LOD, ng/mL	20.3	19.6	20.2	20.3	19.6	20.1	7.51	17.6	25.2	24.8	22.1	7.51	11.2	23.2
Cv_w, %	0.431	1.50	3.39	1.87	2.80	1.13	0.695	3.67	0.917	3.83	1.17	0.427	0.916	3.07
Within-day variability, %	0.0707	1.02	2.05	1.69	2.47	2.97	1.98	2.69	4.60	0.141	0.919	3.18	0.778	0.424
Between-day variability, %	—	1.28	—	—	1.58	—	0.945	1.23	2.05	1.70	—	1.48	—	—
Recovery, %	102.6	98.0	96.4	102	99.2	105	98.2	102	93.7	96.5	97.1	98.8	98.1	103

[a] Molar ratio of ionophore.

Table 3
Potentiometric selective coefficients for the SDM electrodes with solid contact.

Interfering species	$K^{POT}_{SDM^-,J^-}$
Carbonate	−1.35 ± 0.166
Chlorate	−0.233 ± 0.0784
Chloride	−1.52 ± 0.119
Chromate	−0.0813 ± 0.0264
Cyanide	−0.951 ± 0.0376
Fluoride	−1.03 ± 0.107
Hydrogenocarbonate	−1.94 ± 0.0251
Nitrate	−1.22 ± 0.0438
Nitrite	−0.451 ± 0.0727
Persulphate	−0.136 ± 0.0177
Phosphate	−0.454 ± 0.0981
Salicylate	−0.278 ± 0.0633
Sulphate	−2.71 ± 0.106

Table 4
Potentiometric determination of SDM in aquaculture water.

Sample	Taken (ng/mL)	Found (ng/mL)	Recovery (%)
No. 1	19.9	19.3 ± 0.334	96.8
	34.4	34.3 ± 0.630	99.9
	149	147 ± 2.40	97.6
No. 2	19.9	19.2 ± 0.136	97.2
	34.4	34.6 ± 0.781	98.7
	149	150 ±2.11	101

macrocyclic binds sulphonamide antimicrobial drugs [22] and it was therefore selected as a possible ligand, for concentrations of 1×10^{-2}, 1×10^{-4} and 1×10^{-6} mol/L (Table 2). The obtained results showed however that the presence of b-CD did not affect the electrode response, with all concentrations tested (ISE IV–VI) promoting similar features to the equivalent device without it (ISE II).

3.5. Addition of an interfering species

Because the ion-exchange with interfering ions at the inner membrane side becomes important under the limiting condition of very low primary ion concentrations [26], a suitable interfering species at a suitable concentration could favour the potentiometric response. To identify suitable interfering species, the selectivity evaluation of this ISE was conducted for a wide range of possible interfering species.

The anionic species selected to carry out the selectivity study were not only those present in environmental waters (because these may affect the analytical application of the device) but also those that are expected to exert a high interference on the potentiometric response. Carbonate, chlorate, chloride, chromate, cyanide, fluoride, hydrogenocarbonate, nitrate, nitrite, persulphate, phosphate, salicylate and sulphate were considered for this purpose. Potentiometric selectivity coefficients were obtained by the matched potential method, calculated by equation 3. In this method, the potentiometric selectivity coefficient is the activity (concentration) ratio of primary (A) and interfering (B) ions that give the same potential change under identical conditions [27]. At first, a known activity δa_{A^g} of the primary ion solution is added to a reference solution which contains a fixed activity (a_A) of primary ion and the corresponding potential change (DE) is recorded. Thereafter, a solution of interfering ion is added (a_B) to the reference solution a_{A^g} until the same potential change (DE) is recorded.

Table 5
Other methods for SDM determination in water and wastewater.

Type	Detection	Experimental details			Analytical data			Ref.
		Sample pre-treating	Stationary phase	Mobile phase	Linear range[a], 1g/L	LOD[a], 1g/L	Response time, min.	
LC	UV/Vis, 265 nm	LPME, 1-octyl-3-methylimida-zolium hexafluorophosphate, tri-*n*-octylphosphine oxide	C_{18} (150×4.6 mm, 5]m particles)	Acetonitrile and phosphate buffer (pH 5,5)	1–2000	0.1–0.4	'"9	[28]
LC	MS (ESI)	SPE, dichloromethane and acetone	C_{18} (150×2.1 mm, 5]m particles)	Formic acid in water, methanol and acetonitrile	0.5–25	0.005–0.091	<30	[29]
LC	MS/MS (ESI)	SPE, dichloromethane and methanol	C_{18} (150×2.1 mm 3]m particles)	Water and acetonitrile, both in formic acid	0.00001–0.5	0.00003–00033	<15	[30]
LC	MS/MS (ESI)	SPE, acetonitrile and water	C_{18} (150×3.1, mm 3,5 particles)	Formic acid in water and acetonitrile (pH 2.2)	0.00027–0.168	0.0005–0.0002	<16	[31]
LC	MS/MS (ESI)	SPE, ethanol and acetone	C_{18} (150×2.1, mm 3.0]m particles)	Water and acetonitrile, both in formic acid	—	0.00001–0.00784	<12	[32]
LC	MS/MS (ESI)	SPE, water and methanol	C_{18} (150×2,1 mm 3.5]m particles)	Methanol and formic acid in water	0.0012–0.0317	0.001–0.003	<12	[33]
LC	MS/MS (ESI)	SPE, ammonium acetate SPME, ammonium acetate/methanol	C_{18} (250×2.1 mm 5]m particles)	Ammonium acetate in formic acid (pH 3) or ammonium acetate in acetonitrile and methanol	0.020–1.0	—	—	[34]
CE	UV/Vis, 264 nm	SPE, extraction with ammonia and 60% methanol	Fused silica	(150]m×64.5 cm)	Phosphate buffer (pH 7.3) and methanol	75–100	0.23–0.48	—
[35]								
CE	UV/Vis, 265 nm	—	Fused silica (75]m×64.5 cm)	Sodium phosphate and methanol (pH 7.3)	5–250	2.6–23	—	[36]
CE	DAD	SPE, acetonitrile	Fused silica (50]m×96 cm comp.)	Ammonium acetate and ammonium hydroxide (pH 9.5)	5.5–10,000	5.5–65.4	28	[37]

[a] Includes several sulphonamides besides SDM, tested when in LC or CE methods in the same run. LC: Liquid Chromatography; CE: Capillary electrophoresis; MS: Mass spectrometry; ESI: Electrospray ionization SPE: solid-phase extraction; LPME: liquid-phase microextraction; DAD: Diode Array Detector.

$$K_{A,B}^{POT} = \frac{(a_A - a_A)}{a_B} \quad (2)$$

Table 3 lists the obtained potentiometric selectivity coefficients for all previously indicated anions. Almost all logarithm selectivity coefficients were below −0.08, thus illustrating the good selectivity of the membrane. Still, the ionic species with higher potentiometric selectivity coefficients were selected to test the effect of an interfering species inside the inner compartment of the electrode.

Thus, the inner reference solution of 1×10^{-8} mol/L in SDM was added to 1×10^{-4} mol/L in chromate, persulphate, salicylate or chlorate (ISEs VII, VIII, IX and X, respectively). Overall, the obtained results showed that the LOD was strongly dependent on the kind of interfering species present, with a relative order of chromate < persulphate < salicylate "' chlorate. This relative order was also consistent with the relative interfering profile obtained, with chromate being the higher interfering anion, followed by persulphate.

The concentration of chromate in the inner reference solution was studied after changing it from 1×10^{-3} to 1×10^{-6} mol/L (ISEs XI, XII, XIII and XIV, respectively). The obtained results showed that the best analytical performance was achieved for a chromate concentration of 1×10^{-4} mol/L, leading to an LOD of 7.51 ng/mL and an average slope of −54 mV/decade. Higher and lower chromate concentrations depreciated the performance of the ISE, mainly in terms of slope and LOD (Table 2).

3.6. Response time, reproducibility and recoveries of the ISEs

The response times of the electrodes measured the time required to reach emf values within ±1 mV of the final equilibrium potential after immersion in SDM solutions of different concentrations. The maximum time required to reach a steady potential was "'2 min.

Regarding reproducibility, the emf of the electrode at a SDM concentration of 1.6×10^{-6} mol/L was checked five times, between 5 consecutive calibrations. Only small potential variations were observed between these, in all cases < 1.0 mV. The recoveries obtained for the ISEs with SDM standards in buffer suggested the good accuracy of the analytical readings, varying from 92.2% to 98.8% (Table 2).

3.7. Analysis of aquaculture waters

The applicability of the SDM electrodes was checked by testing SDM in aquaculture waters. Since the collected waters contained no SDM, a specific amount of drug was introduced in these samples. Good agreement was found between added and found amounts of SDM. The results of the potentiometric analysis showed recoveries ranging from 96.8% to 101% (Table 4) while the relative error ranged from −0.67 to 3.5% with an average relative standard deviation of 1.2%. The *t*-Student and *F* tests indicated no significant statistical differences between the means of claimed and potentiometric amounts for both skipped samples and the different concentration ranges. The calculated value (*p*) for the *t student* was 0.89 and *F* value 1.0. Both *p* values were below the tabulated critical figures ($t_{critical} = 2.0$ and $F_{critical} = 5.1$) for a 95% confidence level, demonstrating that there are no significant differences between claimed and found amounts (see Table 4).

4. Conclusions

The fabrication of ISEs with a remarkably low detection limit for an organic compound was made possible by optimizing most experimental variables leading to reduced membrane ion fluxes. This included the kind/amount of ingredients in the selective membrane and the kind/amount of compounds in the inner reference solution. Only variations in this last parameter allowed very low limits of detection to be attained, implying that a liquid-contact configuration seemed obligatory for screening drugs spread throughout the environment in the ng/mL concentration range. The use of a micropipette tip as electrode body material made this device a very easy unit to be constructed, and readily available.

Its further comparison to other methods is not easy because only separative methods are found in the literature. This includes liquid chromatography and capillary electrophoresis. The main details of these methods and the corresponding analytical features may be seen in Table 5. In terms of analytical operation, the delectability of the separative approaches is better, but the analytical readings take longer and the experimental procedure requires sample pre-treating stages. Furthermore, the equipment involved is by far more complex and inappropriate to carry out on-site analysis.

In general, the ISE produced here offered high simplicity in design, good precision and a very low limit of detection. The proposed method is simple and inexpensive and could compete with the many sophisticated methods currently available.

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