Sulphonamide-imprinted sol-gel materials as ionophores in potentiometric transduction

S.A.A. Almeida, F.T.C. Moreira, A.M. Heitor, M.C.B.S.M. Montenegro, G.G. Aguilar, M.G.F. Sales

ABSTRACT

This work proposes different kind of solid-contact graphite-based electrodes for the selective determination of sulphonamides (SPHs) in pharmaceuticals, biological fluids and aquaculture waters. Sulfadiazine (SDZ) and sulfamethoxazole (SMX) were selected for this purpose for being the most representative compounds of this group. The template molecules were imprinted in sol-gel (ISG) and the resulting material was used as detecting element. This was made by employing it as either a sensing layer or an ionophore of PVC-based mem- branes and subsequent potentiometric transduction, a strategy never reported before. The corresponding non- imprinted sol-gel (NISG) membranes were used as blank. The effect of plasticizer and kind/charge of ionic lipo- philic additive was also studied.

The best performance in terms of slope, linearity ranges and signal reproducibility and repeatability was achieved by PVC membranes including a high dielectric constant plasticizer and 15 mg of ISG particles. The corresponding average slope was −51.4 and −52.4 mV/decade, linear responses were 9.0×10^{-6} and 1.7×10^{-5} M, and limits of detection were 0.74 and 1.3 μg/mL for SDZ and for SMX, respectively. Good selectivity with log Kpotb−0.3 was observed for carbonate, chloride, fluoride, hydrogenocarbonate, nitrate, nitrite, phosphate, cyanide, sulfate, borate, persulphate, citrate, tartrate, salicylate, tetracycline, ciprofloxacin, sulphamerazine, sulphatiazole, dopamine, glucose, galactose, cysteine and creatinine. The best sensors were successfully applied to the analysis of real samples with relative errors ranging from −6.8 to +3.7%.

Keywords

Sulfadiazine, Sulfamethoxazole, Sol–gel, Molecularly-imprinted, Potentiometry

1. Introduction

Sulphonamide (SPH) drugs were the first effective chemotherapeutic agents employed for preventing bacteria from synthesizing folic acid, an essential chemical to their growth and are among the most used antibiotics in aquaculture activities [1]. Their similarity with the structure of *p*-aminobenzoic acid (PABA, a key ingredient in bacterial synthesis of folic acid), makes bacteria to mistakenly convert the drug instead of PABA into folic acid, thus inhibiting the normal growth of the microorganism.

Due to the development of resistance in formerly susceptible microorganisms, only a few sulpha drugs are used today, among which sulfadiazine (SDZ) and sulfamethoxazole (SMX) (Fig. 1). SDZ is used to treat toxoplasmosis as well as to prevent certain types of meningococcal meningitis [2]. SMX has been used successfully for the treatment of bacterial infections, including those of the respiratory and urinary tract, as well as in the treatment of opportunistic infections in transplantation and for AIDS related complications [3]. They have been employed also as an antimicrobial in aquaculture environment, posing serious risks of food and environmental contamination [2], and subsequent drug resistance episodes [4].

Most methods for SPH determination in pharmaceutical samples require capillary electrophoresis with UV [5] or electrochemical detection [6], and optical [7-9] and electrochemical methods [10-12]. Other methods for SPH determination are meant for the analysis of food or feeding stuffs [13-15], and biological fluids [16]. Few methods have been devoted to environmental samples [17-20], and these concern complex and high cost pre-sampling procedures, unsuitable for in situ or for routine control of waters such as those in the fish tanks.

Ion-selective electrodes (ISEs) could be an alternative to the previous methods, because they offer high precision and rapidity, low cost of analysis, and enhanced selectivity and sensitivity over a wide range of concentrations [21,22]. The binding between the ionophore and the target ion is the molecular-level phenomenon, sensed by an ISE [23]. Therefore, the ISE selectivity towards several ions is regarded to derive from the difference in the binding strengths between the selected ionophore, to be used in the sensor, and the different ions.

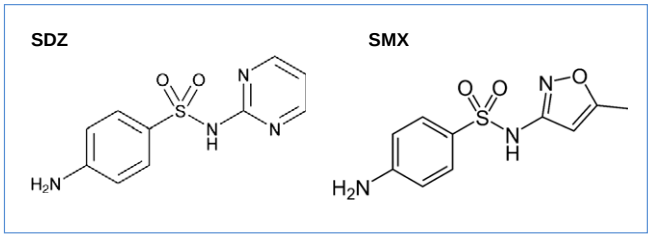

Fig. 1. Binding isotherm (A) and Scatchard plot (B) for ISG particles. Q is the amount of ISG bound to 15.0 mg of polymer; t = 25 °C; V = 10.00 mL; binding time: 20 h.

The ionophore or the ion carrier is thus a vital component in the selective membrane [23]. Ion exchangers and neutral macrocyclic compounds have been employed with success for this purpose over the past decades. However, the design of new sensing materials that are complementary to the size and charge of a particular ion may lead to very selective interactions [24-26]. This could be done by imprinting the target analyte (SDZ and SMX) on a solid surface [27,28].

Molecular imprinting (MI) allows the preparation of synthetic polymers with specific binding sites for a target molecule. This can be achieved if the target is present during the polymerization process, thus acting as a molecular template [29]. The template molecule is removed without disturbing the geometry of the solid matrix and the molecularly-imprinted polymer (MIP) keeps the ability to rebind it. Template rebind produces electrical, optical or mass changes in the nanostructured sensor, thus enabling its detection [30]. Essentially, there are two main routes to produce MIP structures: polymerization of vinyl monomers carrying different chemical functions or sol-gel technology, a useful method to prepare glass-like or ceramic materials through the hydrolysis and condensation of suitable metal alkoxides [31].

The sol-gel process can be described as the construction of an oxide network by progressive polycondensation reactions of molecular precursors in a liquid medium. This technique is currently gaining importance [32] because it is a rather convenient and simple way to incorporate heat sensitive compounds in porous ceramic materials [33]. It also permits designing new materials with tailor-made pore sizes and shapes for specific analyte recognition. Inorganic materials prepared by a sol-gel process have been implemented into a variety of technologies, including nonlinear optical devices, luminescent solar concentrators, and chemical sensors. Still, the application of imprinted sol-gel materials to the production of potentiometryic sensors is in its infancy [34,35].

Thus, this work proposes the construction of SDZ and SMX selective electrodes based on ISG material. The ISG is also ground and used as electroactive material on PVC SDZ and SMX selective membranes. Non-imprinted sol-gel (NISG) materials were used as a blank control of the imprinting process. The sensors were evaluated in steady-state conditions and applied to the analysis of drugs, water and biological samples.

2. Experimental

2.1. Apparatus and electrodes

All potential measurements were made by a Crison μpH 2002 decimilivoltammeter, at room temperature, and under 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 ways out, enabling the simultaneous reading of six ISEs. The assembly of the potentiometric cell was as follows: conductive graphite | SDZ selective membrane | buffered sample solution (HEPES, 1×10^{-3} M, pH 5.0) | | electrolyte solution, KCl | AgCl(s) | Ag. The reference electrode was an Orion Ag/AgCl double-junction (Orion 90-02-00). The selective electrodes were prepared in conventional configuration, with no internal reference solution and an epoxy-graphite solid contact [36].

2.2. Reagents and solutions

All chemicals were of analytical grade and de-ionized water (conductivity ϸ 0.1 μS cm^{-1}) was employed. SDZ, SMX, p-*tert*-octylphenol (TOP), tetraoctylammonium bromide (TOABr), tetraphenylborate (TPB), o-nitrophenyloctyl ether (oNPOE), bis(2-ethylhexyl)sebacate (bEHS), dibutyl phthalate (DBP), poly (vinyl chloride) (PVC) of high molecular weight, 3-aminopropyltriethoxysilane (APTES), diphenyldimethoxysilan (DPTS), tetraethyl orthosilicate (TEOS) were purchased from Fluka. Methanol (MeOH) and tetrahydrofuran (THF) were obtained from Riedel-deHäen. Buffer solutions were 0.001 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). Interference of other chemicals was evaluated for 1.2×10^{-4}, 5.0×10^{-4}, and 1.0×10^{-3} M solutions prepared in buffer.

2.3. Synthesis of host-tailored sol–gel material and preparation of ISG membrane electrode

The water/alkoxide ratio was set to 3, ensuring a transparent smooth surface without cracks and with suitable porosity for the intended purposes (higher ratios decrease the thickness, shrinkage, and pore volume [37] of the matrix). ISG polymers were obtained by dissolving 12.5 mg of each SPH in 1.5 mL of APTES and 1.5 mL of DPTS, acting as functional monomers, in 4.0 mL of methanol. This mixture was stirred for 30 min at room temperature. Then TEOS (0.5 mL), the reticulating agent, was added, followed by hydrochloric acid (0.1 M, 0.3 mL) and desionized water (2.5 mL) to produce the alkoxysilan hydrolysis. The sol was stirred for a further 7 h until a viscous solution was achieved. The conductive graphite surface of the electrodes was immersed into this solution for 7 s. The membranes obtained were dried at room temperature for 2 days. NISG sensor was prepared in a similar way, by excluding the template from the procedure.

2.4. Preparation of the membrane electrodes

Sol-gel membranes were obtained by coating a clean graphite support [25] with 200 μL of the viscous ISG solution. This solution was allowed to dry at room temperature. NISG electrodes were prepared similarly and acted as control of the imprinting effect.

PVC membranes were prepared by including ISG particles inside the PVC matrix. For this purpose, the solid ISG material was grounded and sieved to particle sizes ranging 50−150 μm. SPH was removed from the ISG particles by washing them several times in water (the absence of SPH was confirmed by controlling the absorbance of the washout solution at 260 nm; the particles were repeatedly washed until SPH was no longer detected). The polymer was dried after at 60 °C under vacuum until constant weight. Variable amounts of ISG particles for SDZ were then mixed with 350 mg of plasticizer (*o*NPOE, bEHS or DPB) (Table 1). Some membranes were also added of TOABr, TOP and TPB, acting as additive. The sensor solution was added to 210 mg of PVC previously dissolved in 5.0 mL THF. These membranes were applied over graphite conductive supports. Membranes were let dry for 24 h. NISG PVC membranes were prepared similarly as control. SMX selective membranes were prepared in parallel for some compositions, following similar procedures.

2.5. Potentiometric procedures

All potentiometric measurements were carried out at room temperature. Emf values of each electrode were measured in solutions of fixed pH and ionic strength. Increasing concentration levels of SPH were obtained by transferring 0.02-10.0 mL aliquots of

Table 1
Composition SDZ selective electrodes of imprinted sol-gel and membrane sensors, main analytical features in 1×10^{-3} mol L^{-1} HEPES buffer, pH 5.0.

Characteristic	ISE I	ISE II	ISE III	ISE IV	ISE V	ISE VI	ISE VII	ISE VIII	ISE IX	ISE X	ISE XI	ISE XII	ISE XIII
Sensing material (SM)	MIP/ISG	MIP/ISG	NIP/ISG	MIP/PVC	MIP/PVC	NIP/PVC	MIP/PVC	MIP/PVC	MIP/PVC	MIP/PVC	MIP/PVC	MIP/PVC	MIP/PVC
Amount of SM, mg	-	-	-	15.0	15.0	15.0	15.0	15.0	15.0	15.0	5.0	10.0	30.0
Additive	-	TOP	-	-	TOP	-	-	-	TOABr	TPB	-	-	-
Plasticizer	oNPOE	oNPOE	oNPOE	oNPOE	oNPOE	oNPOE	bEHS	DPB	oNPOE	oNPOE	oNPOE	oNPOE	oNPOE
Slope, mV/decade	−46.3 ± 1.5	−51.8 ± 1.5	−44.2 ± 3.2	−51.4 ± 1.8	−43.5 ± 1.1	−32.3 ± 2.0	−50.9 ± 1.6	−43.3 ± 0.0	−60.2 ± 0.3	−12.0 ± 1.9	−41.6 ± 1.3	−39.6 ± 0.1	−42.6 ± 3.9
R^2 (n= 3)	0.993	0.992	0.996	0.992	0.992	0.995	0.994	0.992	0.992	0.995	0.998	0.995	0.991
Cv_w (%)	6.1	7.3	3.2	8.0	0.8	1.0	5.3	0.0	1.8	9.1	4.4	4.4	6.4
LOD, μg/mL	5.6	6.5	14	0.74	4.0	6.5	17	11	0.87	4.2	2.4	1.2	3.1
LOD, mol/L	2.0×10^{-5}	2.4×10^{-5}	4.9×10^{-5}	2.7×10^{-6}	1.5×10^{-5}	2.4×10^{-5}	6.3×10^{-5}	4.0×10^{-5}	3.2×10^{-6}	1.5×10^{-5}	8.9×10^{-6}	4.5×10^{-6}	1.1×10^{-5}
LLLR, μg/mL	19	9.0	14	2.5	9.0	11	4.4	25	4.1	21	3.3	4.4	6.4
LLLR, mol/L	7.0×10^{-5}	3.3×10^{-5}	5.0×10^{-5}	9.0×10^{-6}	3.3×10^{-5}	4.0×10^{-5}	1.6×10^{-5}	9.0×10^{-5}	1.5×10^{-5}	7.6×10^{-5}	1.2×10^{-5}	1.6×10^{-5}	2.4×10^{-5}

LOD: Limit of Detection, LLLR: Lower Limit of Linear range; R^2: Squared correlation coefficient; SD: Standard deviation; Cv_w: Reproducibility.

1.0×10^{-3} M SPH aqueous solutions to a 100 mL beaker containing 50.0 mL of 1.0×10^{-3} M of buffer. Potential readings were recorded after stabilization to ±0.2 mV and emf was plotted as a function of logarithm SPH concentration. Calibration graphs were used for subsequent determination of unknown SPH concentrations.

2.6. Binding affinity assays between template/host molecules

Binding experiments were carried out by placing 15.0 mg of ISG washed particles in contact with 8.0 mL of SPH solution with varying concentrations ranging 0.4−4 mM. The mixtures were shacked for 12 h at room temperature and the solid phase separated by centrifugation (3000 rpm, 10 min). The concentration of free SPH in the supernatant was also detected by UV spectrophotometry at 260 nm. The amount of SPH bound to the polymer was calculated by subtracting the concentration of free SPH from the initial SPH concentration. The data obtained was used for Scatchard analysis [38].

2.7. Determination of SDZ and SMX in serum, aquaculture water and pharmaceuticals

Potentiometric analysis was carried out on oral dosage forms of pharmaceutical preparations, commercially available as Broncodiazina® (Vitoria, Portugal), with a labeled amount of 3.6 g SDZ per 100 mL. This suspension was dispersed in water by sonication and diluted in buffer to a concentration within the linear range.

For the analysis of aquaculture water or biological fluids, an amount of 250 μl of water or serum were placed in a 25 mL volumetric flask and diluted to final volume with 1.0×10^{-3} M HEPES (pH 5.0). The direct potential method was applied to determine SDZ and/or SMX in all samples.

3. Results and discussions

In the sol-gel process, a sol is first formed by mixing a liquid alkoxide precursor, water, a co-solvent (usually ethanol or methanol) and a catalyst (acid or base) at room temperature [39]. Through continuous monomer hydrolysis and condensation reactions, a porous gel network is thus obtained. Gel aging and drying can be conducted under controlled conditions in order to obtain dense solid matrices. The sol-gel route provides a useful method to prepare glass-like or ceramic materials through the hydrolysis and condensation of suitable organically modified precursors and permits the creation of an oxide network by progressive polycondensation reactions of the molecular precursors in alcoholic medium.

APTES was selected in this study to copolymerize with TEOS because an amine function would ensure a positive electrostatic environment in the sol-gel layer under sufficiently acidic media. This feature may enhance the sensitivity to negatively charged compounds, such as SDZ or SMZ in aqueous media. The selectivity of this material to a specific compound was attributed by MI technology. This was made by preparing the sol-gel in the presence of the template and removing it afterwards by suitable washout procedures.

3.1. Rebinding properties of ISG particles

Binding experiments were carried out by incubating fixed amounts of ISG particles with different concentrations of template until equilibrium was reached. The resulting binding capacity of ISG particles was calculated according to the following equation:

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

where Q is binding capacity of ISG (μmol/g), C_i the initial SPH concentration (μmol/mL), C_f the final SPH concentration (μmol/mL), V_s the volume of solution tested (mL), M_{ISG} the mass of dried ISG particles (mg).

The binding capacities were plotted against the initial SPH concentration. The adsorption data showed that the binding capacity of particles increased with the increasing of the initial concentration of SPH, reaching saturation for higher concentrations.

The binding data were further processed with Scatchard analysis, using the equation

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

where Q was the binding capacity; C_{free} the free analytical concentration in equilibrium (μM); Q_{max} the maximum apparent binding capacity; and K_D the dissociation constant at binding site. The equilibrium dissociation constant was calculated from the slopes and the apparent maximum number of binding sites from the *y*-intercepts in the linear plot of Q/C_{free} versus Q. The Scatchard plots showed a tendency to a linear behavior, suggesting the existence of a single kind of binding site in ISG particles. The corresponding equilibrium dissociation constants K_d for dry polymers were 1319 and 1410 μM for SDZ and SMX, respectively.

3.2. Physical features of the polymer

SEM analysis evidenced the formation of thick films without cracks, and with porous and rough structure. Images suggested that the thickness of the film was 846.1 nm and the diameter of the pore ranged approximately 27 and 52 nm (Fig. 2). The swelling of the membrane was about 3% in mass gain when in contact with water

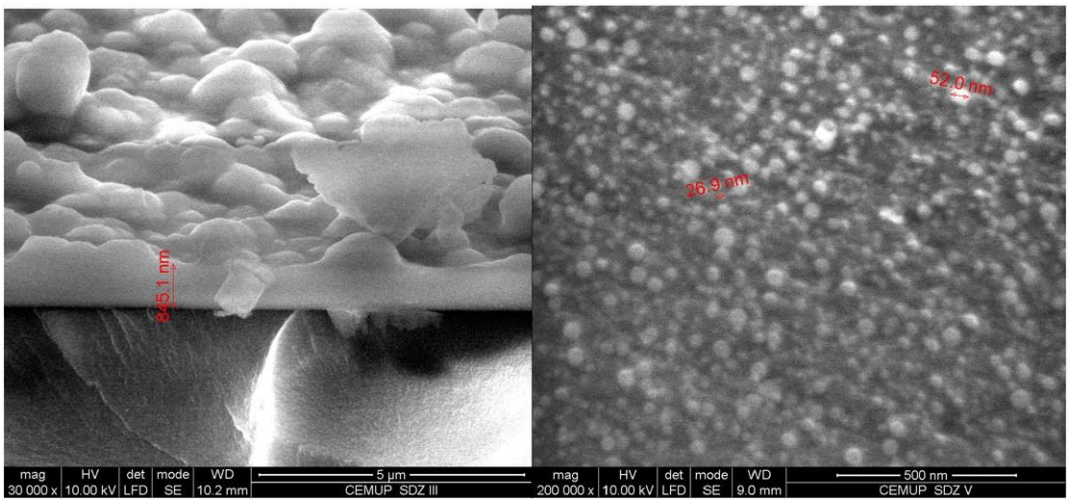

Fig. 2. SEM images of ISG polymer.

before calibration. FTIR spectra were made for grounded sol-gel matrix by an ATR accessory. The spectra displayed intense absorption band of ether bonds at about 1023 cm^{-1} (Fig. 3).

3.3. Sensors performance

The analytical data obtained for all ISEs is indicated in Tables 1 and 2. The reported limits of detection and linear behavior displayed relative standard deviations below 10%.

First, sol-gel membranes were prepared with imprinted (ISE I) or non-imprinted (ISE III) matrices. The ISG sensors displayed linear responses after 7.0×10^{-5} and 4.0×10^{-5} M, average anionic slopes of −46.3 and −49.3 mV/decade and detection limit of 5.6 and 3.1 µg/mL, respectively. The NISG sensors displayed a similar behavior, as may be seen in Fig. 4. This pointed out that the analyte was recognized by general electrostatic interactions with the silica groups of the sol-gel matrix. The possibility of improving the behavior by adding an additive was tested also by preparing sol-gel membranes including TOP (ISE II). This additive has an acid-base character, being mostly anionic or neutral. Under acidic conditions the molecule is essentially neutral with strong positive polarization on the hydrogen atom. This additive improved the analytical performance of the sensors in terms of slope and detection limit (Fig. 4A). Thus, it seems that the analyte was attracted to the membrane by hydrogen bonds [40], favoring the interaction of the anionic SPH with the hydrophobic membrane interface.

Similar electrodes to ISEs I to III were prepared by including the same materials in a PVC matrix (ISEs IV to VI), in order to check the influence of the membrane material on the performance of the sensors. This was done by doping PVC membranes with about 5% of the same ISG material that was finely pulverized. MIP/PVC ISEs showed some improvements in terms of limit of detection, decreasing from 5.6 to 0.74 µg/mL for SDZ (Table 1) and 3.1 to 1.3 µg/mL for SMX (Table 2). The imprinting effect was only evident here, with PVC based membranes. NISG PVC membranes displayed lower slopes than the corresponding ISG membranes; when a sol-gel layer was

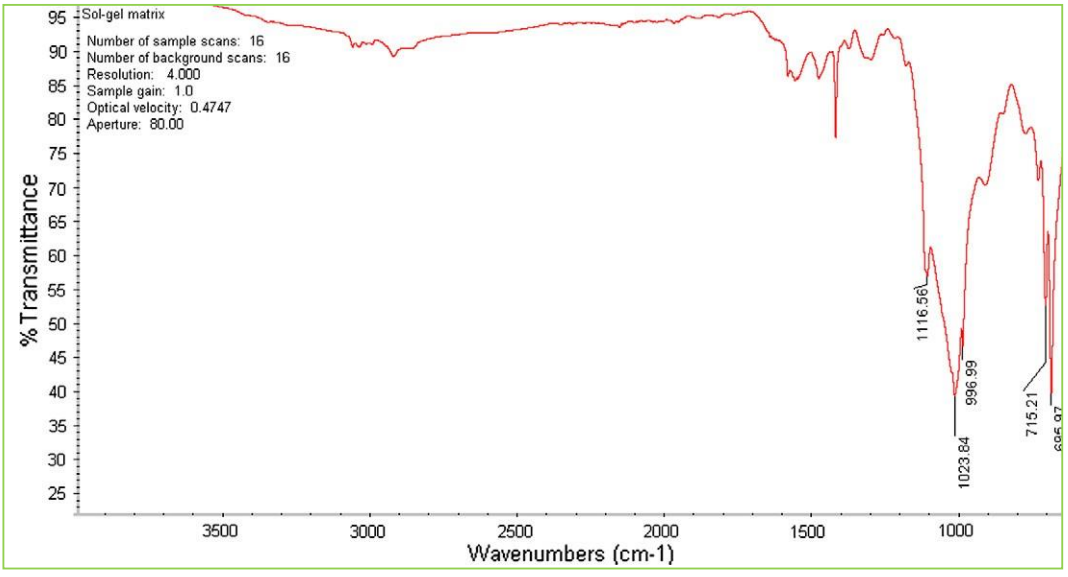

Fig. 3. FTIR spectra of the sol-gel matrix.

Table 2
Composition SMX selective electrodes of imprinted sol-gel and membrane sensors, main analytical features in 1×10^{-3} mol/L HEPES buffer, pH 5.0.

Characteristic	ISE I	ISE II	ISE III	ISE IV	ISE VI	ISE VII	ISE IX
Sensing material (SM)	MIP/ISG	MIP/ISG	NIP/ISG	MIP/PVC	NIP/PVC	MIP/PVC	MIP/PVC
Amount of SM, mg	–	–	–	15.0	15.0	15.0	15.0
Additive	–	TOP	–	–		–	TOABr
Plasticizer	oNPOE	oNPOE	oNPOE	oNPOE	oNPOE	bEHS	oNPOE
Slope, mV/decade	-49.3 ± 0.9	-51.1 ± 0.2	-41.9 ± 0.4	-52.4 ± 0.6	-36.1 ± 1.1	-51.9 ± 0.3	-59.5 ± 1.4
R^2 (n= 3)	0.993	0.996	0.999	0.996	0,995	0.996	0.997
Cv_w (%)	1.8	0.32	0.91	1.2	3.1	0.61	2.4
LOD, μg/mL	3.1	2.6	3.9	1.3	3.8	1.7	1.5
LOD, mol/L	1.2×10^{-5}	1.0×10^{-5}	1.6×10^{-5}	5.1×10^{-6}	1.5×10^{-5}	6.7×10^{-6}	6.1×10^{-6}
LLLR, μg/mL	10	8.4	13	4.3	13	5.1	5.1
LLLR, mol/L	4.0×10^{-5}	3.3×10^{-5}	5.0×10^{-5}	1.7×10^{-5}	5.0×10^{-5}	2.0×10^{-5}	2.0×10^{-5}

used no significant differences were observed between imprinted and non-imprinted versions. The TOP additive was not found relevant in the present case.

The use of PVC implicated the presence of a plasticizer material, for which different kinds of plasticizers were tested: oNFOE, bEHS and DBP, corresponding to ISEs IV, VII and VIII. This test was only conducted for SDZ and suggested that higher dielectric constant mediating solvents favored the potentiometric response produced by the selective membrane. oNFOE was found the best plasticizer (Fig. 4B), with the corresponding electrodes displaying the lower limits of detection.

The effect of additive in PVC-based membranes was also tested (Fig. 4C) and, as in the previous studies, this test was only conducted for SDZ membranes. TOP, TOABr and TPB were used for this purpose, corresponding to ISEs V, IX and X. Typically, the addition of cationic compounds of lipophilic nature to potentiometric sensors reduces the anionic interference and lowers the electrical resistance of the membranes [41]. TOABr was selected for this purpose (Table 1). The corresponding sensors showed Nernstian responses but the limit of detection was higher than that obtained for the equivalent membrane without additive. When TOP was used as additive instead, no improvements were observed. This compound is not ionized within the membrane but is able to establish hydrogen-bonds to the target analyte. The benefits provided by a positively charged additive were confirmed by using an additive of opposite charge in the membrane. The observed analytical response in this case was hindered, which was most probably a result of the anionic exclusion role played by the membrane.

The amount of ISG particles used as ionophore in the PVC membranes was also tested from 5 to 30 mg. The results pointed out that 15 mg was required to achieve higher slopes. Lower and higher values displayed narrower linear ranges and higher limits of detection.

3.4. Response time and lifetime

The time required to achieve a steady potential response (±3 mV) using the proposed sensors in 10^{-6} to 10^{-4} M SDZ or SMX solutions with a rapid 10-fold increase in concentration was ƀ 15 s. Replicate calibrations for each sensor indicated low potential drift, long-term stability and negligible change in the response of the sensors. The sensors were stored and conditioned in 10^{-6} M SDZ or SMX solution of pH 5.0. With all sensors examined, the detection limits, response times, linear ranges and calibration slopes were reproducible within ±3% of their original values over a period of at least 1 week for ISG sensing surface or at least 2 months for PVC membranes.

3.5. Sensor selectivity

Selectivity tests were conducted for ISEs I, III and IV, with the purpose of observing the effect of the sol-gel matrix and the imprint effect. ISE IX was also included as it was the best electrode found in this work. Selectivity was assessed by means of potentiometric selectivity coefficients (K^{POT}), calculated by the mixed solution method (MSM). The logarithmic values of log K^{POT} were indicated in Fig. 4. These were calculated by means of Eq. (3),

$$K^{POT}_{SPH,J} = a_{SPH}/\left(a_J\right)^{Z_{SPH}/Z_J} \quad (3)$$

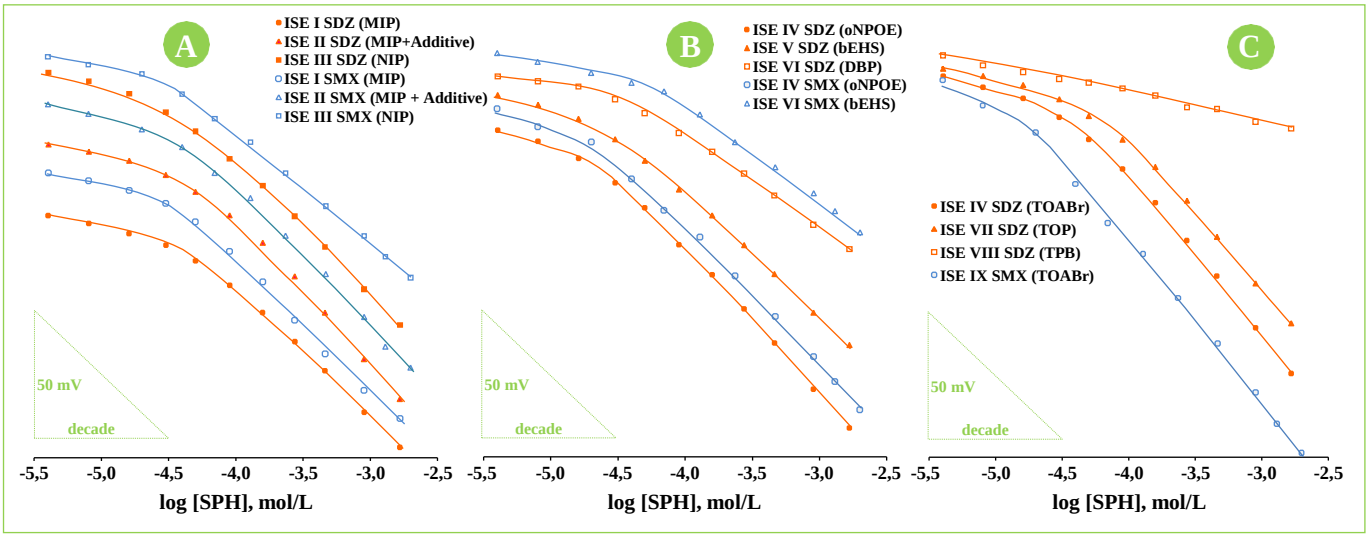

Fig. 4. Calibration plots of sol-gel and PVC membranes (A) with different plasticizers (B) and additives (C).

where a_J is 5.0×10^{-3} M of interfering species, Z the ionic charges of main and interfering ions and a_{SPH} the intersection of the extrapolated linear portions of the plot emf versus the logarithm of SPH concentration. In general, the values of log K^{POT} showed the degree of preferential interaction for SPH over different ionic species. Compounds that are commonly present in pharmaceuticals, biological or water samples were considered for this purpose.

A wide range of inorganic anions was tested, including carbonate, chloride, fluoride, hydrogencarbonate, nitrate, nitrite, phosphate, cyanide, sulfate, borate, persulphate, citrate, tartrate, and salicylate. These included species whose control in water is obliged and that may be present in biological fluids and pharmaceuticals. The obtained values were indicated in Table 3. In general, the results showed negligible interference in both SDZ and SMX selective electrodes, with log K^{POT} values lying within a narrow range: −0.20 to −3.9 and −0.29 to −3.8 for SDZ and SMX selective electrodes, respectively.

Several organic compounds were also selected, such as the antibiotics tetracycline, ciprofloxacin, others SPHs, namely sulphamerazine and sulphatiazole, dopamine, glucose, galactose, cysteine and creatinine. The selectivity of the ISEs against these species were evaluated only for SDZ because this was the only drug marketed alone (without trimethoprim) for human use. Negligible interference was also observed for all electrodes.

Overall, there was no evident difference between the observed behaviors of ISG or PVC based membrane electrodes.

3.6. Monitoring SDZ and SMX in serum, urine, drugs and aquaculture waters

To assess the applicability of the proposed method to real samples, commercial drug (Broncodiazine® syrup, Vitoria, Portugal), serum and aquaculture waters were analyzed for SDZ and SMX contents. Only SDZ was tested for pharmaceuticals and biological samples, given that the use of SMX is not that common in this context or is not done alone. The real samples were spiked when no SPH was found. The analysis was carried out after calibration procedure. To assess the reliability of the results, each sample was analyzed in triplicate.

The potentiometric results are given in Table 4. In general, the proposed sensors for SDZ and SMX operated suitably under laboratory conditions. The precision of the analysis of both SPHs were good and similar (Table 4). The relative standard deviations were always below 5%; for SDZ and SMX electrodes these results ranged from 0.6 to 1.4% and from 0.2 to 3.9%. A satisfactory agreement was obtained between found and claimed amounts. The mean recovery of the amounts taken in the proposed method ranged from 99.7 to 103.7, 93.2 to 94.8 and 97.4 to 105.6% for pharmaceuticals, serum samples and aquaculture waters, respectively. The corresponding relative errors varied from −0.3 to 3.7, −6.8 to −5.2 and 0.0 to 5.7%.

The *t*-student test confirmed that there were no significant differences between the means of claimed and potentiometric amounts of both SPHs (Table 4). The calculated t values (p) were 0.73 and 0.77 for SDZ and SMZ. In both cases, p values were below the tabulated critical t (2.36 and 2.20, respectively) for a 95% confidence level, demonstrating that there are no significant differences between the claimed and the potentiometric amounts.

4. Conclusions

ISG technique was employed to produce SDZ and SMX host-tailored sensors for potentiometric transduction. The sol-gel imprinted layer was used first as sensory surface and compared later to PVC-based sensors carrying the same imprinted material as ionophore. The best performance was obtained by ISG particles dispersed in PVC. A cationic additive offered some improvements in terms of analytical performance, although it was not essential to reach a near-Nernstian response. The sensors were found appropriate for a practical application to real samples.

Advantages of these sensors include the simplicity in designing, low cost, quick response, reproducible and repeatable data, low limit of detection and good selectivity. It also offers the possibility of high analytical trough output by adaptation to flow injection conditions. The proposed method is simple, cheap, precise, accurate and inexpensive regarding reagent consumption and equipment involved.

Table 3
Average potentiometric selectivity coefficients of SDZ and SMX membrane sensors.

Interfering species	$K_{SPH-,J}^{-POT}$							
	ISE I	ISE III	ISE IV	ISE IX	ISE I	ISE III	ISE IV	ISE IX
	SDZ	SDZ	SDZ	SDZ	SMX	SMX	SMX	SMX
CO_3^{2-}	−1.5 ± 0.16	−1.1 ± 0.065	−1.6 ± 0.067	−1.7 ± 0.044	−1.6±0.059	−1.3 ± 0.032	−1.5 ± 0.071	−1.6 ± 0.034
Cl^-	−1.7 ± 0.078	−1.8 ± 0.024	−1.9 ± 0.045	−1.9 ± 0.077	−1.9±0.022	−1.8 ± 0.054	−1.9 ± 0.063	−1.9 ± 0.046
F^-	−1.5 ± 0.11	−1.5 ± 0.045	−1.7 ± 0.034	−1.7 ± 0.075	−1.7±0.027	−1.6 ± 0.075	−1.8 ± 0.034	−1.9 ± 0.057
HCO_3^-	−1.6 ± 0.026	−1.3 ± 0.092	−1.7 ± 0.096	−1.7 ± 0.062	−1.8±0.024	−1.7 ± 0.056	−1.7 ± 0.042	−1.7 ± 0.043
NO_3^-	−0.95±0.037	−0.85 ± 0.14	−1.3 ± 0.12	−1.4 ± 0.065	−1.3±0.043	−1.2 ± 0.087	−1.4 ± 0.012	−1.6 ± 0.069
NO_2^-	−1.02±0.10	−0.99±0.064	−1.4 ± 0.076	−1.5 ± 0.041	−1.5±0.037	−1.3 ± 0.053	−1.4 ± 0.026	−1.8 ± 0.071
PO^{3-}	−3.9 ± 0.025	−3.8 ± 0.083	−3.7 ± 0.046	−3.4 ± 0.091	−3.8±0.012	−3.6 ± 0.013	−3.8 ± 0.069	−3.4 ± 0.039
CN^-	−1.2 ± 0.043	−1.3 ± 0.044	−1.2 ± 0.065	−1.2 ± 0.068	−1.3±0.019	−1.2 ± 0.027	−1.0 ± 0.091	−1.2 ± 0.035
SO^{2-}	−0.51±0.072	−3.3 ± 0.036	−3.2 ± 0.032	−3.5 ± 0.054	−3.2±0.023	−3.4 ± 0.017	−3.5 ± 0.010	−3.4 ± 0.051
Borate	−2.9 ± 0.017	−3.0 ± 0.015	−2.9 ± 0.086	−3.0 ± 0.043	−3.0±0.034	−3.1 ± 0.012	−2.9 ± 0.018	−3.0 ± 0.024
Persulphate	−0.41±0.95	−0.37±0.098	−0.45 ± 0.12	−0.32 ± 0.10	−0.42±0.11	−0.38±0.058	−0.37±0.011	−0.29±0.022
Citrate	−2.8 ± 0.063	−2.6 ± 0.033	−2.9 ± 0.034	−2.5 ± 0.067	−2.2±0.032	−2.7 ± 0.026	−2.5 ± 0.11	−2.7 ± 0.014
Tartrate	−2.7 ± 0.10	−2.5 ± 0.053	−2.4 ± 0.039	−2.7 ± 0.073	−2.5±0.028	−2.7 ± 0.031	−2.5 ± 0.048	−2.5 ± 0.043
Salicylate	−1.7 ± 0.071	−1.9 ± 0.032	−1.7 ± 0.052	−1.7 ± 0.031	−1.7±0.014	−1.7 ± 0.039	−1.9 ± 0.026	−1.6 ± 0.087
Ciprofloxacin	−0.70±0.025	−0.30±0.023	−0.20±0.026	−0.40±0.013	–	–	–	–
Creatinine	−1.0 ± 0.063	−0.90±0.041	−0.60±0.057	−0.89±0.022	–	–	–	–
Cysteine	−1.2 ± 0.032	−0.45±0.049	−0.52±0.085	−0.30±0.037	–	–	–	–
Dopamine	−1.1 ± 0.044	−0.65±0.078	−0.50±0.091	−1.7 ± 0.093	–	–	–	–
Galactose	−1.0 ± 0.034	−0.90±0.066	−0.30±0.034	−1.5 ± 0.083	–	–	–	–
Glucose	−1.3 ± 0.012	−1.5 ± 0.071	−1.4 ± 0.043	−1.5 ± 0.042	–	–	–	–
Sulphamerazine	−1.1 ± 0.038	−1.5 ± 0.014	−0.70±0.047	−0.50±0.013	–	–	–	–
Sulfathiazole	−0.50±0.027	−0.81±0.013	−0.50±0.039	−0.53±0.045	–	–	–	–
Tetracycline	−0.90±0.033	−0.90±0.046	−0.58±0.029	−0.30±0.038	–	–	–	–

Table 4
Potentiometric determination of SDZ and SMX in commercial drugs, biological samples and aquaculture water.

Sample	Drug	Concentration (μg/mL)		Relative	Recovery
		Claimed	Found	Error (%)	(%)
Broncodiazine®	SDZ	25	25.9 ± 0.2	3.7	103.7
	SDZ	50	49.9±1.8	−0.3	99.7
serum	SDZ	50	47.4±2.1	−5.2	94.8
	SDZ	100	93.2±3.3	−6.8	93.2
Aquaculture water	SDZ	35	34.7 ± 0.14	0.8	99.2
(Sample 1)	SDZ	100	105.9 ± 4.20	5.7	105.7
Aquaculture water	SDZ	35	35.2 ± 0.49	0.6	100.6
(Sample 2)	SDZ	100	101.5 ± 0.20	1.2	101.2
Aquaculture water	SDZ	35	35.4 ± 0.71	1.2	101.2
(Sample 3)	SDZ	100	100.2 ± 1.61	0	100
Aquaculture water	SDZ	35	34.1 ± 0.62	2.6	97.4
(Sample 4)	SDZ	100	98.5 ± 2.97	1.8	98.2
Aquaculture water	SMX	35	35.5 ± 0.21	1.5	101.5
(Sample 5)	SMX	100	99.2 ± 0.58	1.1	99.2
Aquaculture water	SMX	35	36.3 ± 0.28	3.7	103.7
(Sample 6)	SMX	100	98.0 ± 1.34	2.2	97.8
Aquaculture water	SMX	35	36.0 ± 0.21	3.0	103
(Sample 7)	SMX	100	100.6 ± 0.59	0.3	100.3
Aquaculture water	SMX	35	36.2 ± 0.35	3.4	103.4
(Sample 8)	SMX	100	99.4 ± 1.36	0.8	99.2

The wastewaters generated by this experimental work are of small concern to the environment regarding its volume and composition.

Acronyms

APTMS	3-aminopropyltrimethoxysilane
bEHS	bis(2-ethylhexyl)sebacate
C_{free}	free analytical concentration
DBP	dibutyl phthalate
DPTS	diphenyldimethoxysilan
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
ISEs	ion-selective electrodes
ISG	imprinted sol-gel
K_d	dissociation constant
K^{POT}	potentiometric selectivity coefficients
MIP	moleculary imprinted polymer
MSM	mixed solution method
NISG	non-imprinted sol-gel
oNPOE	o-nitrophenyl octyl ether
PABA	*p*-aminobenzoic acid
Q	binding capacity
SDZ	sulfadiazine
SEM	scanning electron microscope
SMX	sulfamethoxazole
SPH	sulphonamide
TEOS	tetraethyl orthosilicate
THF	tetrahydrofuran
TOABr	tetraoctylammonium bromide (TOABr)
TOP	p-*tert*-octylphenol
TPB	tetraphenylborate

Acknowledgments

The authors acknowledge the financial support from FCT, Fundação para a Ciência e Tecnologia/FEDER by means of project PTDC/AGR-AAM/68359/2006 and the PhD grant (SFRH/BD/42509/2007) of the first author SAAA.

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