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Sulfadiazine-Potentiometric Sensors for Flow and Batch Determinations of Sulfadiazine in Drugs and Biological Fluids

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New PVC membrane electrodes for the determination of sulfadiazine (SDZ) are presented. The electrodes are fabricated with conventional and tubular configurations with a graphite-based electrical contact, and no internal reference solution. The selective membranes consist of bis(triphenylphosphoranilidene)ammonium·SDZ (electrode A), tetraoctylammonium bromide (electrode B), or iron(II)-phthalocyanine (FePC) (electrode C) electroactive materials dispersed in a PVC matrix of *o*-nitrophenyl octyl ether (*o*-NPOE) plasticizer. The sensors A, B, and C displayed linear responses over the concentration ranges 1.0×10^{-2} – 1.0×10^{-5}, 1.0×10^{-2} – 7.5×10^{-6}, and 3.2×10^{-2} – 7.0×10^{-6} mol l^{-1} (detection limits of 1.09, 2.04 and 0.87 μg ml^{-1}) with anionic slopes of –57.3 ± 0.1, –46.7 ± 0.5, and –65.1 ± 0.2 mV $decade^{-1}$, respectively. No effect from pH was observed within 4.0 – 5.5, 4.8 – 10, and 4.5 – 8, respectively, and good selectivity was found. The sensors were applied to the analysis of pharmaceuticals and biological fluids in steady state and in flow conditions.

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Introduction

Less than a century ago, infectious diseases were the leading causes of death in the world. Sulfonamide drugs were the first effective chemotherapeutic agents employed for preventing bacteria from synthesizing folic acid, a chemical that was essential to their growth. The similarity of the structures of these drugs to the structure of *p*-aminobenzoic acid (PABA, a key ingredient in bacterial synthesis of folic acid), makes bacteria to mistakenly convert the drug into folic acid instead of PABA, thus inhibiting the normal growth of the microorganism. Due to the development of resistance in formerly susceptible microorganisms, only a few sulfa drugs are used today, among which is sulfadiazine (SDZ, chemical structure in Fig. 1). It is used to treat toxoplasmosis as well as to prevent certain types of meningococcal meningitis. Its association with other drugs such as trimetropim (TMTP) enhances the effectiveness of chemotherapy and widens its range of application.

Administration of SDZ in humans or animals is performed by means of suitable pharmaceutical preparations. A rigorous and routine control of its quantity must be established, for which expeditious, simple and inexpensive methods would be appreciated. Only a few works are described in the literature for this purpose. They regard specifically capillary electrophoresis with UV,[1] or electrochemical detection,[2] optical methods such as UV[3,4] or visible[5-7] spectrophotometries, and amperometry.[8] The United States Pharmacopoeia (USP) proposes a chromatographic separation before UV quantification of SDZ.[9] Other methods for SDZ determination are meant for the analysis of food[10-20] or feeding stuffs,[21] biological fluids,[22-27] or environmental samples.[28-31] These concern more complex and high cost procedures, unsuitable for the routine control of formulated pharmaceuticals.

Ion-selective electrodes (ISEs) have found vast applications in diverse fields of analysis.[32-34] They offer high precision and rapidity, low cost of analysis, enhanced selectivity and sensitivity over a wide range of concentrations.[35] In addition, they are easy to construct and manipulate and no sample pretreatment is needed before the analysis itself. Short response times, in the order of seconds, make ISEs appropriate devices for process control.

In the present work, several PVC membrane selective electrodes are described for SDZ determination in batch and in flow injection (FI) evaluations. Each membrane incorporated an ion-exchanger or an anion carrier to provide a selective response to SDZ. The results showed that each individual sensor may be used to prepare an ISE for the drug. Applications of the proposed electrodes for the analysis of commercial pharmaceuticals and biological fluids are described.

Experimental

Apparatus

Measurements were carried out with the electrochemical cell AgCl(s)/Ag double junction reference electrode//test solution/

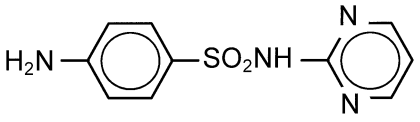

Fig. 1 Chemical structure of SDZ.

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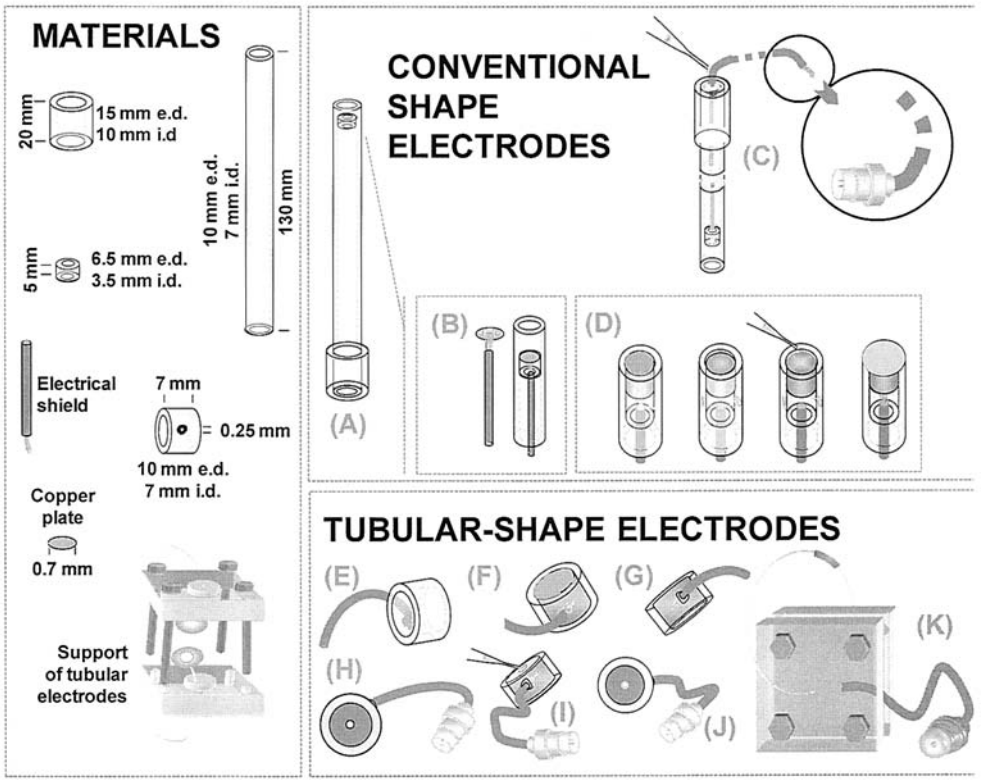

Fig. 2 Construction of conventional and tubular shape SDZ selective electrodes made of Perspex® tubes with a shielded electrical cable using a copper plate as electrical contact to a graphite-based conductive support. A, Electrode body with mounted tubes; B, electrical connection to the copper plate placed in the electrode body; C, attachment of electrical cable and connection to a BNC antenna connector; D, graphite-epoxy conductive support with a drilled cavity (≈1.0 mm depth) and membrane applied over it; E and F, inner cavity filled with graphite-epoxy conductive matrix that fixes the electrical cable inside; G, polished and isolated external surfaces; H, internal hole drilled (≈1.0 mm ϕ); I, application of selective membrane; J, membrane attached to the walls of the inner hole; K, tubular electrode placed in a flow support (closed circuit for membrane conditioning).

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® μpH 2002 decimilivoltammeter (±0.1 mV sensitivity). The analytical output signal was transferred to a commutation point that enabled the reconnection of the signal to one of six ways out. Each way presented an electrical antenna connector that provided suitable adaptation to one selective electrode. The selective electrodes had no internal reference solution and used an epoxy-graphite matrix as conductive solid contact.[36] Batch trials were carried out with electrodes of conventional configuration, and flow ones with electrodes of tubular shape. General procedures of construction may be seen in Fig. 2. The pH values were measured by means of a Crison® CWL/S7 combined glass electrode connected to a decimilivoltammeter Crison® pH meter, GLP 22. All potential measurements were performed under constant stirring, by a Crison® microST 2038. Accurate addition of standard solution was performed by means of Gilson pipetman micropipettes of 100, 200, 1000 and 5000 μl maximum capacities.

The flow injection analysis (FIA) system manifold consisted of a two-channel Ismatech MSREGLO Model peristaltic pump. The manifold (Fig. 3) was connected with polyethylene tubing (Tygon, 0.7 mm i.d.) and an Omnifit injection valve (Rheodyne, Model 7125) with a sample loop of 300 μl volume. The potential signals were recorded using a homemade high-impedance data acquisition 8-channel box connected to a PC

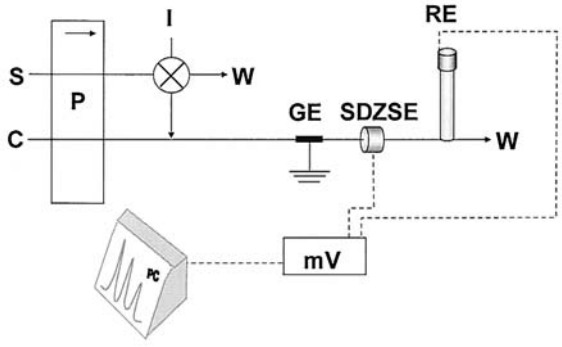

Fig. 3 Schematic diagram of the flow injection system. P, Peristaltic pump; S, sample; C, IS and/or pH adjuster solutions; I, injection valve; GE, grounding electrode; SDZSE, SDZ selective electrode; RE, reference electrode; W, waste; mV, decimillivoltammeter; PC, computer recording.

through the interface ADC 16 (Pico Technology, UK) and PicoLog for windows (version 5.07) software.

High performance liquid chromatographic (HPLC) measurements were made with an SYKNM Chromatograph equipped with an S1100 solvent delivery system, an S2000 HPLC controller, and a Machery-Nagel CC250/14 Nucleosil 150-5 C18 column and a variable wavelength spectrometer LINEAR U-Vis 200. This system operated with a flow-rate of

1.45 ml min^{-1}, an injection volume of 20 µl, and detection at 205 nm. All solutions were degassed in a Fungi lab ultrasonic bath.

Reagents and solutions

All chemicals were of analytical grade and deionized water (conductivity < 0.1 µS cm^{-1}) was used throughout. Sodium SDZ (Na·SDZ) and iron(II)-phthalocyanine (FePc) were purchased from Sigma. *o*-Nitrophenyl octyl ether (*o*-NPOE), tetraoctylammonium bromide (TOAB), tetraphenylborate sodium (TPB), and poly(vinyl chloride) (PVC) of high molecular weight were purchased from Fluka. Bis(triphenylphosphoranyliden) ammonium (BTPPIA) chloride and tetrahydrofuran (THF) were purchased from Aldrich and Riedel-deHaën, respectively. Pharmaceutical preparations were purchased from local drug stores.

The SDZ standard solution for calibrating the potentiometric cell was 2.20×10^{-2} mol l^{-1}. It was prepared by dissolving about 0.3000 g of Na·SDZ in 50.00 ml of water. The ionic strength (IS) was adjusted to 1.0×10^{-2} mol l^{-1} by means of a 3.3×10^{-3} mol l^{-1} Na_2SO_4 solution. Simultaneous pH and ionic strength adjustments were carried out with buffer solution of suitable pH and 10^{-2} mol l^{-1} IS.

The mobile phase of the chromatographic procedure was water/acetonitrile/acetic acid (87:12:1). Standard solutions were prepared in sodium hydroxide 0.025 mol l^{-1}. All solutions were degassed and filtered before use.

ISE preparation

The anion exchanger BTPPIA·SDZ, was prepared by precipitation reaction between 50.0 ml of a 1.0×10^{-2} mol l^{-1} Na·SDZ aqueous solution and 100.0 ml of a 1×10^{-2} mol l^{-1} BTPPIA chloride aqueous solution. The resulting solid was filtered and thoroughly washed with water. It was dried before use under a nitrogen atmosphere and kept in a dark flask inside a desiccator, in order to prevent light and humidity effects.

Membrane solutions were prepared by dissolving about 2% (w/w) of the electroactive materials (BTPPIA·SDZ, TOAB and FePC) in about 65% (w/w) of *o*-NPOE and 33% (w/w) of PVC in *ca.* 3 ml of THF. Successive aliquots (200 µl) of each membrane were placed into a conductive support of graphite and epoxy resin,[36] of conventional or tubular shape (Fig. 2). The selective sensors so obtained will be further addressed as types A, B, and C, according to the sensor solution they contain. An additional sensor, D, was also prepared by the addition of TPB to the FePC based sensing system. All sensors were preconditioned by soaking overnight in a 1×10^{-3} mol l^{-1} SDZ solution and stored in deionized water between daily measurements.

Potentiometric procedures

Calibration curves were carried out following the Litre beaker method.[37] All electrodes were placed in a convenient support over a magnetic stirrer and immersed in 50.00 ml of IS or pH and IS adjuster solution for about 10 min. Suitable increments of a 2.2×10^{-2} mol l^{-1} SDZ standard solution were added. The potential readings of the stirred SDZ solutions were measured at room temperature and recorded after stabilization to ±0.2 mV. A calibration plot was constructed connecting logarithm concentration with electromotive force.

The study of the pH effect was made by dipping the electrodes in 200.0 ml of a 5.0×10^{-4} mol l^{-1} SDZ standard solution prepared in an IS adjuster. Concentrated sulfuric acid solution was added until the pH of 3.0 was reached. Small amounts of a saturated sodium hydroxide solution were later added to provide a pH increase up to pH 11. A second potentiometric cell consisting of a combined glass electrode connected to another decimilivoltammeter monitored the pH of this solution. Operational pH ranges were defined for maximum potential variations of ±6 mV.

The selectivity coefficients were determined by the separated solutions method.[38] The emf values of SDZ or interfering species solutions were measured separately and the corresponding selectivity coefficients were calculated in $\log K^{POT}_{SDZ,J^{z-}}$, using Eq. (1).

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

where E_1 and E_2 are the electrode potentials in 6.0×10^{-4} 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 $decade^{-1}$.

With neutral compounds where the charge (Z) equals zero, Eq. (1) was simplified by cancelling out the term $(1 - (-1/Z))\log C_{SDZ}$.

Flow measurements

The flow setup is depicted in Fig. 3. A series of portions (100 µl) of SDZ test solutions covering the range 1.0×10^{-2} – 1.0×10^{-6} mol l^{-1} was injected into a flow stream of 10^{-2} mol l^{-1} IS adjuster or suitable buffer, flowing at 2.2 ml min^{-1}. The FePC membrane-based sensor was used as a working sensor against a Ag/AgCl double junction reference electrode. Each solution was measured in triplicate. The average potentials at maximum heights were plotted against log[SDZ].

Determination of SDZ in pharmaceutical preparations

Three forms of commercial preparations were analyzed: Tribrissen® 48% injection containing 40 g of SDZ and 8 g of TMTP per 100 mg of formulated product; Broncodiazina® syrup with 3.60 mg of SDZ per 100 mg; and Baclise® 600, with 500 mg of SDZ and 100 mg of TMTP per tablet. Stock solutions of all commercial samples were prepared by placing 90.0 µl of injection, 500 mg of syrup or 450 mg of tablet (from 10 powdered tablets, previously weighed) into 50.00 ml Erlenmeyer flasks. Enhanced dissolution of SDZ was ensured by adding small amounts of NaOH before completing to final volume with buffer. Because small solid residues resulting from the preparation of tablets did not affect the measurements, filtering procedures were omitted. Final SDZ solutions were about 2.9×10^{-3} or 1.5×10^{-3} mol l^{-1}. Potentiometric analysis was carried out after diluting 2500 or 5000 µl of sample solutions to 50.00 ml with buffer. Concentrations expected were of 1.5×10^{-4} mol l^{-1} in SDZ. The SDZ potentiometric cell was calibrated before analysis itself with SDZ standard solutions ranging from 2.0×10^{-4} to 2.0×10^{-3} mol l^{-1}, and prepared in buffer.

Analytical results of pharmaceuticals were compared with those obtained by following USP procedures.[9] Instead of using single standard readings as proposed by USP, calibration curves were established for standard solutions ranging from 1.0×10^{-5} to 7.5×10^{-5} mol l^{-1} prepared in 0.025 mol l^{-1} NaOH.

These were injected into the HPLC system, operating with a C18 column, a UV detector (254 nm), and an eluent of water/acetonitrile/glacial acetic acid (87/12/1) flowing at 2 ml/min. Suitable amounts of sample were diluted/dissolved in 0.025 mol l^{-1} NaOH solution in order to fit the calibration curve. Prior to injection, all sample solutions were filtered and degassed.

Determination of SDZ in human urine

An aliquot of 1.0 ml of human urine sample was diluted with 10^{-2} mol l^{-1} phosphate buffer of pH 5 in a 100 ml calibrated flask and the contents were shaken well. A 5 – 10 ml portion of the diluted urine solution was transferred into a 25-ml beaker.

Table 1 Response characteristics of SDZ selective electrodes of different electroactive materials ($n = 6$)

Parameter	ISE A (BTPPIA·SDZ)	ISE B (TOAB)	ISE C (FePC)	ISE D (FePC + TPB)
Slope/mV decade^{-1}	–57.3 ± 0.1	–46.7 ± 0.5	–65.1 ± 0.2	–52.3 ± 0.7
Correlation coefficient ($n = 6$)/r^2	0.9998	0.9988	0.9997	0.998
Lower limit of linear range/mol l^{-1}	1.0×10^{-5}	7.5×10^{-6}	3.2×10^{-6}	4.2×10^{-5}
Detection limit /μg mL^{-1}	1.09 ± 0.2	2.04 ± 0.1	0.87 ± 0.1	7.02 ± 0.3
Response time/s	<10	<10	<20	<20
Working pH range	4.0 – 5.5	4.8 – 10	4.5 – 8	4.5 – 8
Standard deviation, σ,/mV	0.3	0.5	0.4	0.6
Recovery, %	99.1	98.7	99.6	99.1
Repeatability, %	0.3	0.8	0.9	0.6

Working and reference electrodes were immersed, and the potential readings were recorded after reaching the equilibrium response (10 – 20 s) and compared with the calibration plot.

For continuous measurements (FIA), a flow stream of 10^{-2} M phosphate buffer of pH 5 carrier solution was allowed to pass through the flow-cell at a flow rate of 2.2 ml min^{-1}. Successive 100 μl aliquots of the standard SDZ and unknown test sample solutions were injected into the flowing stream. The corresponding potential change was measured and recorded *versus* time. A typical calibration plot was made and used to determine the concentration of the unknown samples.

Results and Discussion

Composition of PVC membranes

SDZ selective membranes have different kinds of electroactive materials. These include anion exchangers of quaternary ammonium salts such as BTPPIA (electrodes A) or TOAB (electrodes B), and an anion carrier phthalocyanine derivative, FePC (electrodes C). All membranes were of plasticized PVC with 33 wt% PVC, 65 wt% *o*-NPOE solvent mediator and 2 wt% of electroactive material. An anion excluder consisting of 0.5 wt% NaTPB was also added to membranes of FePC (electrodes D).

The main analytical features of SDZ selective electrodes are summarized in Table 1; these were evaluated following IUPAC recommendations.[39] Electrodes with the anion carrier FePC presented a super-Nernstian behavior, with average slopes of –65.1 mV decade^{-1}. Linear behavior was observed from 3.2×10^{-6} up to 1.0×10^{-2} mol l^{-1} with a detection limit 0.87 μg ml^{-1}. Addition of NaTPB to the sensing system resulted in poorer analytical features. A great decrease in sensitivity and an increase in the lower limit of the linear range were recorded. With BTPPIA·SDZ and TOAB membrane-based sensors, electrodes exhibited linear behavior with average slopes of –57.3 and –46.7 mV decade^{-1} over the concentration ranges 1.0×10^{-5} to 1.0×10^{-2} and 7.5×10^{-6} to 1.0×10^{-2} mol l^{-1} with detection limits of 1.09 and 2.04 μg ml^{-1}, respectively.

The repeatability of the potential readings for the sensors was examined by subsequent measurements in 1.0×10^{-3} mol l^{-1} of SDZ solution immediately after measuring the first set of solutions at 1.0×10^{-4} mol l^{-1} of SDZ solution. The standard deviations of measuring emf for 5 replicate measurements obtained were <1.1 and 0.8 mV for the solutions of 1.0×10^{-4} and 1.0×10^{-3} mol l^{-1}, respectively. The corresponding % concentration errors associated to these potential variations are indicated in Table 1 and show that the repeatability of potential response of the sensors is good. The response properties of the sensors did not change significantly after two months use.

Response time

The response times of the electrodes were tested by measuring the time required to reach steady potential values within ±1 mV of the final equilibrium potential after immersion in SDZ solutions, each having a 10-fold difference in concentration. The average dynamic response time for FePC based electrodes was 10 and 30 s for concentrations $>10^{-3}$ mol l^{-1} and $\leq 10^{-3}$ mol l^{-1} SDZ, respectively. BTPPIA·SDZ and TOAB based sensors showed similar behavior, yielding stable potentials within 10 to 20 s for concentrations ranging from 1.0×10^{-2} to 1.0×10^{-6} mol l^{-1}. All membrane sensors exhibit day-to-day reproducibility of better than 0.9 mV for 1.0×10^{-2} – 1.0×10^{-6} mol l^{-1} SDZ solutions.

Effect of pH

The influence of pH on the response of SDZ membrane sensors plasticized with *o*-NPOE was checked by recording the emf displayed by a 5.0×10^{-4} mol l^{-1} SDZ standard solution at various pH values. Potential *versus* pH profiles showed that the electrodes did not respond to pH changes in the ranges 4.0 – 5.5 for electrode (A), 4.8 – 10 for electrode (B), and 4.5 – 8 for electrode (C) (Table 1). At pH < 4, SDZ is ionized to a small extent and cannot be sensed as a monovalent anion, leading to potential increases. For pH > 8, a slight decrease in potential was recorded, thus inducing hydroxide interference.

Evaluation of the main operating features for all electrodes under constant pH was carried out in several buffer solutions prepared within the corresponding operational pH ranges. The best results were achieved for 10^{-2} mol l^{-1} IS buffer solutions of H_2SO_4/NH_3 at pH 4.5 for electrode A or phosphate buffer at pH 5 for electrodes B and C.

Selectivity

The selectivity of the chemical sensor is one of the most important potentiometric features. One component of the selective membrane that exerts great influence upon this property is the electroactive material, because the mechanism of selectivity is mainly based on stereospecificity and electrostatic environment. The selectivity of SDZ-selective electrodes was assessed by calculating the potentiometric selectivity-coefficients ($K^{POT}_{SDZ^-,J^{z-}}$) after the separate solution method.[38]

The selectivity of an ion-pair-based membrane electrode depends on the physico-chemical characteristics of the ion-exchange process at the membrane–sample solution interface, on the mobility of the respective ions in the membrane, and on the hydrophobic interactions between the primary ion and the organic membrane.[40] Selectivity coefficients of SDZ membranes with BTPPIA·SDZ and TOAB indicated near-Hofmeister

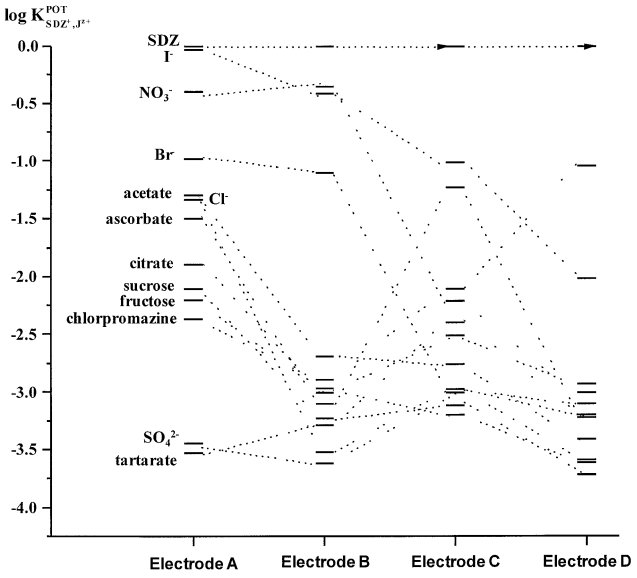

Fig. 4 Potentiometric selectivity coefficients at 6.0×10^{-4} M.

behavior, in which the more lipophilic species are the preferred ones. Overall, the selectivity patterns were SDZ > I^- >NO_3^- > Br^- > acetate > Cl ~ ascorbate > citrate > sucrose > fructose > chlorpromazine > SO_4^{2-} > tartrate, CH_3COO^- > chloride > sulfate = phosphate, and SDZ > NO_3^- > I^- > Br^- > Cl^- > chlorpromazine > citrate ~ fructose > ascorbate > sucrose ~ tartrate > CH_3COO^- > SO_4^{2-}, respectively (Fig. 4).

For metalloporphyrins acting as ionophores, besides the electrostatic interaction between the central metal and anions, there is a selective coordination capability, favoring the interaction of less lipophilic anions.[41] Thus, the selectivity profile can be quite different from the classical ion exchange-type electrodes. For FePC membrane-based sensor, the selectivity pattern was SDZ > I^- > ascorbate > tartrate > chlorpromazine > NO_3^- > citrate > CH_3COO^- > Br^- > sucrose > fructose ~ SO_4^{2-} > tartrate. These results indicate clear deviation from the classical Hofmeister selectivity order, suggesting that the anions were selectively coordinated with the central metal Fe(II) at the membrane/sample interface. The anion exchange at the axial coordination site was essential for the potentiometric response. Indeed, the selective coordination of anions to the metal center should be preceded by the generation of electrophilic sites at the membrane/sample interface through the liberation of initial axial ligands. The addition of anionic (TPB^-) sites to a FePC-based membrane sensor induced limited responses towards all anions and thus enhanced selectivity,[42-44] as shown in Table 2.

Membranes with FePC and TDMAC lead to selectivities similar to those in the Hofmeister series. On the other hand, the addition of anionic sites improves the selectivity for more than one logarithmic unit (see also Table 2). The fact that lipophilic ions of the same charge as the measuring ions improve the ion selectivities is surprising at first glance.

Flow measurements

A tubular-type detector (Fig. 2) incorporating a FePC based membrane sensor was prepared and used under hydrodynamic mode of operation for continuous SDZ quantification. A linear relationship between SDZ concentrations and FIA signals was obtained over a concentration range from 1.0×10^{-5} to 1.0×10^{-2} mol l^{-1} using 10^{-2} mol l^{-1} phosphate buffer, pH 5 (Fig. 5). The slope of the calibration plot was near-Nernstian (–50.3 ± 0.6 mV decade^{-1}). The slightly lower sensitivity of the detector in FIA may be attributed to several factors such as mass transport rate, sample dispersion and effect of contact time between sample and electrode.[45] The limit of detection was 2.18 ± 0.2 μg ml^{-1} and the sampling frequency was 30 to 35 samples per hour.

Table 2 Average potentiometric selectivity coefficients of SDZ membrane sensors

Interfering species	$\log K^{POT}_{SDZ^+,J^{z-}}$			
	BTPPIA·SDZ ISE A	TOAB ISE B	FePC ISE C	FePC + TPB ISE D
I^-	–0.03	–0.41	–1.01	–2.02
NO_3^-	–0.4	–0.35	–2.4	–3.01
Br^-	–0.98	–1.1	–3.01	–3.1
CH_3COO^-	–1.31	–3.52	–2.98	–3.59
Cl^-	–1.32	–2.69	–2.76	–3.41
Ascorbate	–1.5	–3.1	–1.23	–3.22
Citrate	–1.9	–2.97	–2.51	–2.93
Sucrose	–2.11	–3.23	–3.12	–3.72
Fructose	–2.2	–3.01	–3.2	–3.61
Chlorpromazine	–2.37	–2.89	–2.21	–1.04
SO_4^{2-}	–3.45	–3.62	–3.21	–3.71
Tartrate	–3.53	–3.29	–2.11	–3.2

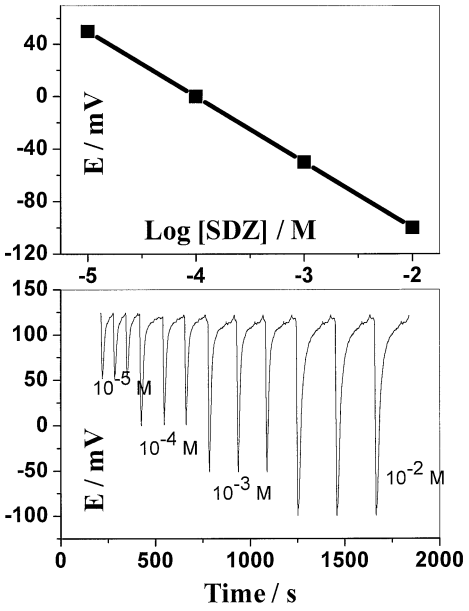

Fig. 5 Typical (FIA) peaks produced by injection of 100 μL aqueous solutions of standard SDZ into a stream of 10^{-2} M phosphate buffer pH 5 flowing at 2.5 mL min^{-1} using the FePC PVC membrane based sensor.

Analytical Applications

In order to access the applicability of the proposed selective electrodes, we applied the methods for the determination of SDZ in its pharmaceutical preparations and in biological fluids.

Assessment of SDZ in pharmaceutical formulations

Determination of SDZ content in its tablets, syrup and injection was carried out with electrodes A. Table 3 gives the mean results obtained with two electrodes on several

Table 3 Analysis of commercial pharmaceuticals by proposed (POT, batch) and reference (USP) methods

Form	Claimed amount	POT[a]	USP[a]	*F*-Test
Tablet[b]	500 mg/tablet	487 ± 22 (4.7%) *n* = 16	497 ± 18 (3.7%) *n* = 12	1.04
Syrup	7.2 g/200 mL	7.10 ± 0.30 (4.6%) *n* = 15	7.24 ± 0.16 (2.3%) *n* = 12	1.88
Injection[c]	40% (w/v)	39.7 ± 1.9 (4.7%) *n* = 13	39.1 ± 2.41 (6.2%) *n* = 12	2.70

a. Mean values, standard deviation and variation coefficient.
b. Contains 100 mg of TMTP.
c. Contains 8% of TMTP.

independent preparations of each lot (*n* in Table 3). Average values obtained varied between 97.4 and 99.3% of labeled amounts. The potentiometric method compared well to the USP method,[9] in which SDZ is determined by HPLC with UV detection. Considering as null hypothesis that the two methods agree, a paired two-tail test for 5% level of significance gave a calculated *t* (0.662) below the tabulated one ($t_{0.025,11}$ = 2.20), therefore accepting the null hypothesis. Comparison of variances attained for each sample was made by the *F*-test using the same assumptions as for the *t*-Student test. Calculated values (Table 3) were always below the critical *F*-value ($F_{0.025(10,10)}$ = 3.72), thus confirming the null hypothesis.

TMTP is often associated to SDZ in formulated products to enhance the effectiveness of the chemotherapy. This was the case of the injection and tablets indicated in Table 3, where relative amounts of TMTP and SDZ were 5:1. Because TMTP in pharmaceuticals is always below, interference from TMTP in the proposed potentiometric method is not expected.

Determination of SDZ in spiked human urine

Clinical pharmacological studies were made previously in infants and in children aged 3 to 49 months. These patients were given 0.3 – 0.4 ml kg^{-1} of suspension containing 41 mg of SDZ. The concentrations in urine at 24 h were about 35 to 40 mg L^{-1}.[46]

To assess the suitability of the proposed potentiometric method to monitor SDZ in human urine, we spiked samples with SDZ levels <15 mg L^{-1}. Results obtained with FePC electrodes using batch and FIA modes are listed in Table 4. In batch experiments, average recoveries and corresponding relative standard deviations were of 96.9% and 0.8%, respectively. For hydrodynamic mode of operation these were 95.6% and 1.3%. SDZ values calculated after potentiometric analysis were linearly regressed against the taken amount in search of fixed or proportional bias. In static and flow modes the equations were $C_{ISE} = 0.9862 \times C_{added} + 0.0411$ (*R* squared = 0.9996) and $C_{ISE} = 0.9421 \times C_{added} + 0.0483$ (*R* squared = 0.9995), respectively. The slopes and the intercepts of the regression lines did not differ significantly from ideal values, suggesting the absence of systematic errors. The *t*-test confirms that there is no significant difference between the means and variances of static and hydrodynamic potentiometric sets of results. The good agreement between the added and found SDZ content in the samples demonstrates the applicability of the sensor for routine analysis without a prior separation.

Table 4 Batch and flow injection potentiometric determination of SDZ in spiked human urine samples (*n* = 6)

Sample No.	Added/μg mL^{-1}	Found/μg mL^{-1}	
		Batch	FIA
1	0.9	0.81 ± 0.1	0.85 ± 0.1
2	1.5	1.39 ± 0.4	1.44 ± 0.2
3	2.5	2.55 ± 0.3	2.39 ± 0.3
4	5.0	4.85 ± 0.2	4.90 ± 0.1
5	10.0	9.82 ± 0.1	9.41 ± 0.5

Conclusions

Proposed SDZ potentiometric detectors are simple, inexpensive and easy to manipulate. The proposed electrodes might be useful detectors in steady state and in flow systems for analysis of pharmaceuticals and biological fluids. They display high selectivity, wide dynamic response range and rapid response. The overall procedure is 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. Aside from dissolution and dilution, no sample pretreatment or separation steps are required. The proposed method also enables high sampling frequencies with low operator intervention, meaning that it is suitable for the routine procedures carried out at pharmaceutical industries and analytical laboratories.

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