



## Cyclodextrin-based potentiometric sensors for midazolam and diazepam

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### ABSTRACT

In this work the implementation of benzodiazepine ion-selective electrodes for pharmaceutical formulations control is described. The solid-contact electrodes for midazolam and diazepam are based on polymeric membranes incorporating respectively  $\beta$ -cyclodextrin and (2-hydroxypropyl)- $\gamma$ -cyclodextrin as ionophores, 2-fluorophenyl 2-nitrophenyl ether as plasticizer and potassium tetrakis (*p*-chlorophenyl) borate as ionic additive. For conventionally shaped midazolam electrode a slope of  $61.9 \pm 1.3$  mV dec<sup>-1</sup>, a LLR of  $5.7 \pm 2.7 \times 10^{-4}$  g L<sup>-1</sup> and pH range of 2.6–5.4 was obtained, while the corresponding values for diazepam electrodes were of  $67.6 \pm 3.0$  mV dec<sup>-1</sup>,  $4.9 \pm 1.5 \times 10^{-2}$  g L<sup>-1</sup> and 1.9–2.7 pH units, respectively. Membrane optimization was based on the molar ratio between the ionophore and additive for midazolam and on inclusion cavity of cyclodextrin for diazepam. The miniaturization of the above-described electrodes gave rise to potentiometric detectors for sequential-injection lab-on-valve system with similar characteristics albeit the useful lifetime shortened from 1 year to approximately 15 days under continuous operation. The optimized flow conditions were achieved for sample injection volumes of 20  $\mu$ L propelled towards the detection cell at the flow rate of 16  $\mu$ L s<sup>-1</sup> during 80 s. Real sample analysis revealed statistical accuracy and between-days precision comparable to the general used chromatographic-based procedure.

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### 1. Introduction

Benzodiazepines are psychoactive therapeutic drugs with varying hypnotic, sedative, anxiolytic, anticonvulsant, muscle relaxant and amnesic properties, which are brought on by slowing down the activity of the central nervous system. Two representative benzodiazepines are midazolam and diazepam. Midazolam (8-chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5*a*][1,4]benzodiazepine) [1] is indicated for the acute management of aggressive or delirious patients and less for the acute management of seizures such as status epilepticus. Occasionally is used as a hypnotic, especially in hospitals.

Diazepam (7-chloro-1-methyl-5-phenyl-3*H*-1,4-benzodiazepin-2(1*H*)-one) [1] is mainly used to treat anxiety, insomnia, and symptoms associated with the acute alcohol or opiate withdrawal. It also induces sedation, anxiolysis or amnesia prior to certain medical procedures. These applications gained popularity among medical professionals, increasing the number of pharmaceutical preparations in the market and consequently a need of development of different technics for quality control.

Spectrophotometric [2,3] and adsorptive stripping voltammetric [4] procedures are indicated in the literature for midazolam determinations in pharmaceuticals. Other techniques has been used for the diazepam determinations such as the direct square-wave (SWV) and square-wave cathodic stripping (SWCSV) voltammetries using the hanging mercury drop electrode [5], spectrophotometry [6,7], capillary gas chromatography with a nitrogen-phosphorus detector [8], reversed-phase liquid chromatography and capillary electrophoresis [9]. The documented potentiometric procedures for diazepam are based on the use of solid-contact-ion-selective electrodes (ISE) with polyvinylchloride membrane supporting an electroactive ion-pair complex, such as tetraphenylborate-diazepam or phosphotungstate-diazepam [10]. The best working characteristics are obtained after previous conditioning for 24 h with an appropriate solution of the analyte. A similar approach was previously described by Nie et al. [11] by proposing ion-pair complexes such as tetraphenylborate-diazepam, dipyridylamine-diazepam, tetraiodobismuthate-diazepam or tetra-iodomercurate-diazepam for the development of selective electrodes with internal reference solution. From these works however several drawbacks could be pointed out to the use of ion-pair as electroactive material. The electrodes presented short lifetimes, they need to be implemented in a configuration that includes the use of an internal reference solution and require a long conditioning time (24 h) in the analyte concen-

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trated solution [10]. Recent theoretical advances on the establishing the response mechanisms of ion-selective electrodes led to the proposal of different species capable of molecular recognition which include criptands, calixarenes, cyclodextrins among others [12]. In this new context, ion-selective electrodes without memory effects, long life time, easy to construct and with very reproducible responses were obtained resorting to cyclodextrins as electroactive material for the determinations of other therapeutical drugs like diclofenac and amantadine [13,14]. Those works revealed it was possible to establish a fast and reversible complexation equilibrium by optimizing the type of cyclodextrin ( $\alpha$ ,  $\beta$ ,  $\gamma$  or modified) supported in the polymeric membrane. Both the ability of cyclodextrins to alberg lipophilic guests in its apolar cavity and the lipophilic character of benzodiazepines could be exploited to achieve new concept based ion-selective electrodes. PVC membranes supporting different types of cyclodextrins were tested in this work to accomplish the determinations of midazolam and diazepam. Furthermore, a previously proposed miniaturized electrode configuration [15] is adopted aiming its coupling to a sequential-injection lab-on-valve system (SI-LOV) in order to allow automatic and miniaturized determinations. Similarly to sequential-injection analysis, SI-LOV technique allows sample and reagent solutions to be selected, mixed and diluted automatically by means of its sequential aspiration from the core stream selecting valve, into a holding coil [16]. Through down scaling benzodiazepines-selective electrode, it was possible to benefit from the advantages recognized in SI-LOV-based systems, namely regarding equipment portability, reduced consumption of sample and reagents and reduction of effluent waste. The reduced volume of the sensor cocktail used in the proposed configuration allows for the preparation of an increased number of electrodes with modest costs. Electrical noise, that is frequently present in potentiometric-based procedures, is significantly reduced.

## 2. Experimental

### 2.1. Reagents and solutions

Distilled, deionised water (conductivity  $<0.1 \mu\text{S cm}^{-1}$ ) and analytical grade chemicals were used without further purification, unless otherwise stated. Carboxylated polyvinyl chloride (PVC-COOH), potassium tetrakis (*p*-chlorophenyl)borate (KTPClPB) were from Fluka; tetrahydrofuran (THF) was from Riedel-de-Haën; 2-fluorophenyl 2-nitrophenyl ether (FNDPE),  $\alpha$ -cyclodextrin ( $\alpha$ -CD),  $\beta$ -cyclodextrin ( $\beta$ -CD), (2-hydroxypropyl)- $\gamma$ -cyclodextrin, and dibutyl phthalate (DBP) were from Sigma. Midazolam and diazepam (Roche, Basle) were kindly offered by Pharmaceutical Technological Laboratory of Faculty of Pharmacy (U.P.).

A stock solution of midazolam, was daily prepared by weighing about 67 mg of reagent into a 100 mL volumetric flask and subsequent dilution to the mark with an acidic buffer solution (HCOOH/NaOH) at pH 3 with an ionic strength adjusted to  $0.01 \text{ mol L}^{-1}$ . The working calibrating solutions were prepared daily by rigorous dilution with the same buffer solution which was further used as carrier in the developed flow system.

A stock solution of diazepam, was prepared daily by weighing about 140 mg of reagent into a 100 mL volumetric flask and subsequent addition of  $160 \mu\text{L}$  of HCl  $6 \text{ mol L}^{-1}$ , followed by the addition of 10 mL of LiCl  $0.1 \text{ mol L}^{-1}$  at pH 2. For complete dissolution the solution was sonicated for 3 min before completing the volume of the flask. The working calibrating solutions were prepared by rigorous dilution with LiCl  $0.1 \text{ mol L}^{-1}$  at pH 2. This solvent was used as carrier during SI-LOV determinations of diazepam.

Oral pharmaceutical samples were obtained from local drug stores. Samples of midazolam tablets (labelled amount of 15 mg/tablet) were prepared by weighing the content of 20 capsules from the same lot and finely powdering in an agate mortar. Afterwards, an aliquot of about 100–120 mg, was dissolved in 25 mL of buffer solution (pH 3;  $I = 0.01 \text{ mol L}^{-1}$ ) in order to fit the expected analyte concentration in the linear range of the studied electrode. To perform the alternative method described by Pfendt et al. [3], based in a spectrophotometric determination, 0.1, 1 and  $5 \text{ mol L}^{-1}$  chloride acid (Merck) solutions were used.

Samples of diazepam were prepared by weighing the content of 20 capsules or tablets from the same lot and finely powdering in an agate mortar. To an amount of 110–250 mg of powder,  $16 \mu\text{L}$  of HCl  $6 \text{ mol L}^{-1}$  and 1 mL of LiCl ( $I = 0.1 \text{ mol L}^{-1}$ ; pH 2) were sequentially added, being the mixture sonicated during 3 min. The volume was completed until 10 mL, with LiCl ( $I = 0.1 \text{ mol L}^{-1}$ ; pH 2), in order to fit the expected analyte concentration in the linear range of the studied electrode. Samples from diazepam ampoules for intravenous injection were also prepared in the same way, but 0.9 mL aliquots of sample were used instead. To perform the reference method proposed by USP Pharmacopoeia [17], based in a liquid chromatographic determination, acetonitrile (Fluka), water and methanol (Fluka) (2:2:1) were used for capsules and extended-release capsules. A mixture of methanol/water (65:35) was used instead for injectable samples.

### 2.2. Apparatus

A Crison 2002 pH potentiometer (sensitivity:  $\pm 0.1 \text{ mV}$ ) coupled to an Orion 605 electrode switcher was used for measuring the potential differences between the Orion 90-02-00 doubled junction AgCl/Ag reference electrode and the benzodiazepine-selective electrodes. The potentiometric signals were recorded with a Kipp & Zonen BD 111 recorder coupled to the decimillivoltmeter. The pH values of all solutions and the operational pH range characteristics of the electrodes were controlled by means of a Phillips GAH 110 glass electrode.

The schematic representation of the computer-controlled SI-LOV system used is depicted in Fig. 2a. It comprises a Minipuls 3 Gilson (Viliers-le-Bell, France) peristaltic pump with a PVC pumping tube ( $\varnothing_{\text{int}} = 0.90 \text{ mm}$ ) of the same brand, a VICI C25-3118E, eight-port stream selecting valve (Valco Instruments, Houston, TX), a 161T031 NResearch three-way solenoid valve (Stow, MA), and a Crison MicropH-2002 potentiometer to which a Metrohm electrode of Ag/AgCl (KCl  $3 \text{ mol L}^{-1}$ ), model 6.0727.000 was connected. Four channels ( $\varnothing_{\text{int}} = 0.5 \text{ mm}$ ) were drilled in a single acrylic block with 20-mm thick in order to respectively access the central and three lateral ports of the selecting valve. In one of these channels a transverse hole with 0.5-mm diameter was drilled in order to screw the reference electrode in a perpendicular position (Fig. 2b). The cyclodextrin electrode is in a wall-jet position in the end of the flow channel. The reference and the cyclodextrin electrodes are in perpendicular position each other. A PTFE coil with 30 cm (HC) and flow lines were made with  $\varnothing_{\text{int}} = 0.5 \text{ mm}$  PTFE tubing. The rotation speed of the peristaltic pump (P), the rotor position of the eight-port valve (MsV) and the solenoid valve (SV) on/off switching were controlled by means of a PCL-711 Advanced interface card coupled to a microcomputer running a software written in Quick Basic 4.5.

The spectrophotometric determinations of midazolam in pharmaceutical formulation for the alternative method were obtained using a Lambda 45 UV/vis spectrophotometer and quartz cells with 1 cm of optical path were used for the comparative procedure in midazolam determinations.

The analysis of the diazepam samples by HPLC was carried out in a Merck Hitachi chromatographic system comprising a model 7100

**Table 1**  
Membrane composition (% w/w) of the constructed electrodes for midazolam and diazepam

Type	KTpCIPB % (w/w)	FNDPE % (w/w)	$\alpha$ -CD % (w/w)	$\beta$ -CD % (w/w)	2HP $\gamma$ -CD % (w/w)	PVC-COOH % (w/w)
I	1.9	67.0		1.4		29.7
II	0.3	68.0		1.2		30.5
III	1.6	63.5		6.4		28.5
IV	0.3	68.3	1.0			30.4
V	0.3	67.7			1.8	30.2

$\alpha$ -CD,  $\alpha$ -cyclodextrin;  $\beta$ -CD,  $\beta$ -cyclodextrin; 2HP $\gamma$ -CD, (2-hydroxypropyl)- $\gamma$ -cyclodextrin; KTpCIPB, potassium tetrakis (*p*-chlorophenyl)borate; FNDPE, 2-fluorophenyl 2-nitrophenyl ether; PVC-COOH, carboxylated polyvinyl chloride.

pump, a Rheodyne 7725i injector (20- $\mu$ L loop) and an Alltech ODS-2 C18 column (250 mm  $\times$  4.6 mm). A diode array system, model 7455 was used as detector and the data processed by means of software package of the same brand, model D7000.

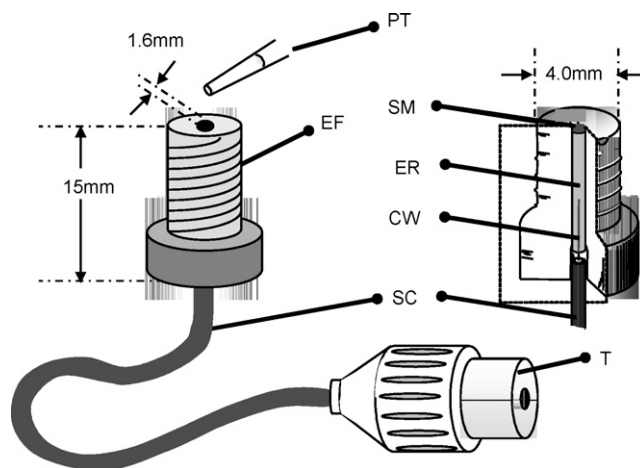
### 2.3. Electrodes membrane preparation and electrode construction

PVC membranes for electrode construction (Table 1) were prepared by mixing different amounts of cyclodextrin and additive in plasticizer solvent. This sensor solution was then added to PVC-COOH previously dissolved in THF. Moreover electrodes with membranes without cyclodextrin or without cyclodextrin plus additive were also implemented.

The membranes were dropped directly on the conductive surface of the electrode, made up with a mixture of epoxy resin (Araldite) and graphite powder, and dried at room temperature for 2 day. The electrodes were soaked in water for 30 min before evaluation. Miniaturized electrodes, based on tube end-fittings as electrode body, were developed afterwards accordingly [15] (Fig. 1). These electrodes were coupled to the LOV (Fig. 2) and the conditioning solution was then flowed through at a flow rate of 9  $\mu$ L s<sup>-1</sup> for 5 min.

### 2.4. Procedures

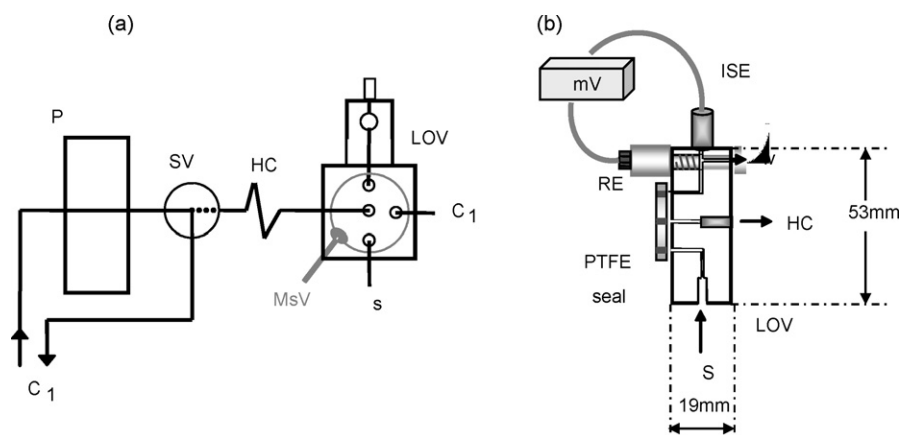
Conventional evaluation of the midazolam electrodes was accomplished by means of several calibrations obtained in a buffer solution (HCOOH/NaOH) at pH 3 with an ionic strength (*I*) of 0.01 mol L<sup>-1</sup> and varying the midazolam concentrations between 5  $\times$  10<sup>-5</sup> and 7  $\times$  10<sup>-1</sup> g L<sup>-1</sup>. The pH influence on the electrodes response was evaluated between pH 2 and 11, at two concen-



**Fig. 1.** Schematic view of the miniaturized benzodiazepine ion-selective electrodes obtained by drop wise 20  $\mu$ L of the sensor solution from a 100  $\mu$ L pipette tip (PT) over the central hole of a commercial end fitting (EF), previously filled with an conductive epoxy resin (ER) in contact with the central wire (CW) of a shielded cable (SC). (SM) sensing membrane; (T) terminal connector to decimillivoltmeter.

tration levels by means of changing the pH of main solution through the addition of NaOH or H<sub>2</sub>SO<sub>4</sub> concentrated solutions. For the diazepam electrodes, calibration curves were obtained in LiCl 0.1 mol L<sup>-1</sup> at pH 2 in the concentration range between 9.0  $\times$  10<sup>-3</sup> and 1.3 g L<sup>-1</sup>. The study of pH influence on the electrodes response was performed in the same way as described above. In the SI-LOV system the potentiometric response was evaluated for different hydrodynamic variables such as flow-rates and sample volume, in order to guarantee analytical signals independent of the sample volume and maximum allowable sampling rate. A measurable and reproducible peak height was obtained for sample injections volumes higher than 20  $\mu$ L with a settled pumping rate of 16  $\mu$ L s<sup>-1</sup>. Then, analyte solutions at different concentrations were driven at 10  $\mu$ L s<sup>-1</sup> during 2 s by the port 2 (sample port in Fig. 2a) and sent towards the flow-through detection cell at a flow rate of 16  $\mu$ L s<sup>-1</sup> during 80 s.

Interference evaluation presented in the sample matrices (like lactose, starch, ammonium chloride, sodium chloride and magnesium stearate) for benzodiazepines samples was performed using the matched potential method (MPM) [18] in order to assess the effect of these interferences in the optimized analytical procedure. The selectivity coefficient in this case is defined as the activity (con-



**Fig. 2.** Schematic view of the proposed set-up. (a) The system comprises a peristaltic pump (P) synchronized with a solenoid valve (SV), a holding coil (HC), an acrylic lab-on-valve (LOV) manifold screwed over the rotor of a stream selecting valve (MsV). (W) waste; (C<sub>1</sub>) carrier solution and (S) sample. (b) Side view of the reference and benzodiazepine electrode screwed on LOV; (RE) reference electrode and (ISE) benzodiazepine ion-selective electrodes.

**Table 2a**  
Potentiometric working characteristics for midazolam ion-selective electrodes

Membrane type	II	II <sup>a</sup>	III	IV
Slope (mV/dec)	61.9 ± 1.3	56.0 ± 1.9	57.4 ± 1.8	62.1 ± 1.3
LLLR (g L <sup>-1</sup> )	(5.7 ± 2.7) × 10 <sup>-4</sup>	(4.2 ± 2.6) × 10 <sup>-4</sup>	(5.8 ± 1.9) × 10 <sup>-4</sup>	(8.5 ± 4.7) × 10 <sup>-4</sup>
PDL (g L <sup>-1</sup> )	(1.3 ± 0.5) × 10 <sup>-4</sup>	(1.6 ± 0.6) × 10 <sup>-4</sup>	(3.7 ± 0.9) × 10 <sup>-4</sup>	(3.9 ± 2.5) × 10 <sup>-4</sup>
C <sub>max</sub>	6.9 × 10 <sup>-1</sup>	6.9 × 10 <sup>-1</sup>	6.9 × 10 <sup>-1</sup>	6.9 × 10 <sup>-1</sup>
Response time (s)	<15	<15	<15	<15
pH	[2.6;5.4]	[2.2;3.5]	[2.0;4.0]	[2.7;4.1]

<sup>a</sup>Electrode with an internal reference obtained after electrochemical polymerization of pyrrol in the presence of potassium hexacyanoferrate(II)/(III).

centration) ratio of the primary ion and the interfering ion which gives the same potential change in a reference solution.

### 3. Results and discussion

#### 3.1. Evaluation of conventionally shaped electrodes for midazolam and diazepam

Several electrodes based on membranes incorporating different cyclodextrins (ionophore) were implemented resorting to 2-fluorophenyl 2-nitrophenyl ether as plasticizer and potassium tetrakis (*p*-chlorophenyl)borate as anionic additive (Table 1) and their characteristics evaluated in batch conditions. Their composition was established after performing some trials considering electrodes with membranes possessing only the plasticizer or plasticizer plus additive. For the first ones erratic potentials were obtained, due to the diffusion of the analyte into the membrane phase due to its lipophilicity. According to the phase-boundary model established for polymeric membrane selective electrodes [19], the potential is determined by the ratio between the activity of the main ion in the aqueous phase to the activity in the membrane phase. Nernstian-response is obtained if the activity of the main ion in organic phase is kept low and constant. In turn, for membranes prepared with plasticizer plus additive a cumulative effect is observed. Meanwhile, for low analyte concentrations the diffusion into membrane phase predominates, for concentrations higher than about 10<sup>-4</sup> mol L<sup>-1</sup> of midazolam an exchange phenomenon took place. However, the response kept very irreproducible. With the incorporation of cyclodextrin the performance of the electrode improved dramatically. To the reduction of the anionic additive amount in the membrane relatively to the ionophore concentration corresponded to obtain linear instead of sigmoid calibration curves. This change occurred by limiting the inward flux of midazolam to guarantee membrane electroneutrality condition. Establishing a molar ratio of 3:2 for CD:ionic additive, a Nernstian-response of 61.9 ± 1.3 mV dec<sup>-1</sup> was found. No improvement was registered from the increase of the amount of both components in the membrane (electrode type III). The membranes based on  $\alpha$ -cyclodextrin presents similar slope (62.1 mV dec<sup>-1</sup>) but slightly higher lower limits of linear range (LLLR) and practical detection limit (PDL) when compared with electrodes based on  $\beta$ -cyclodextrin (Table 2a). All of the described electrodes present responses time lower than 15 s. The effect of pH on the potential was smaller than 5 mV, independent of the midazolam concentration, in the range of 2.6–5.4 (ESI type II). For pH higher than 5 the potential variation registered decrease drastically. This is in accordance with the midazolam pK<sub>a</sub> which is approximately 6. The lifetime of this electrode was longer than a year without any particular conditioning procedure. Any comparison of the performance is possible to establish once potentiometric sensors for midazolam could be found in the literature. Moreover, trials to get both increased stability and improvement of lower detection limit were accomplished by creating a known internal reference potential. Therefore, a con-

trolled redox potential between membrane and the solid contact was implemented through the electropolymerization of pyrrol in the presence of potassium hexacyanoferrate(II)/(III) before depositing the sensor membrane [20] (electrode type II<sup>a</sup>). A lower slope, 56.0 ± 1.9 mV dec<sup>-1</sup>, and a narrow pH plateau range were found.

To accomplish diazepam determinations, the same electrodes were first tried. Reproducible super-Nernstian slopes were obtained (Table 2b), besides some memory effect arriving from diffusion of this specie into membrane, during the calibration. The super-Nernstian response could be related with superimpose of the relative higher lipophilic characteristics of the diazepam to the buffer effect imposed by the presence of the cyclodextrin. Thus the effect of the inclusion cavity of the ionophore used was evaluated by the implementation of a new electrode with a cyclodextrin possessing an higher inclusion cavity (electrode type V). As seen in Table 2b, the general potentiometric response characteristics improved. Besides the slope, the LLLR are two concentration decades higher than the membranes proposed by Nie et al. [11]. However, the useful life of the electrodes is more than a year which is much more extended than the reported ones [10,11]. The operational pH range registered (potential variation smaller than 5 mV) is more reduced when compared to the obtained for midazolam once the diazepam pK<sub>a</sub> which is approximately 3.3.

#### 3.2. Evaluation of the miniaturized electrodes coupled to a SI-LOV system

General working characteristics of the best conventionally shaped electrodes were down-sized to be coupled to the SI-LOV system. Successive calibrations in the range between 4 × 10<sup>-3</sup> and 5 × 10<sup>-1</sup> g L<sup>-1</sup> were then performed using the hydrodynamic conditions described above. Nernstian slopes were found (60.5 ± 0.5 mV dec<sup>-1</sup>) between the concentrations of 2.3 × 10<sup>-3</sup> to 4.4 × 10<sup>-1</sup> g L<sup>-1</sup> of midazolam and of 61.3 ± 2.7 mV dec<sup>-1</sup> in the concentration range of 1.2 × 10<sup>-1</sup> to 1.7 g L<sup>-1</sup> of diazepam. Slightly decrease of the slopes was found comparing with batch evaluation. The practical detection limit (PDL) was of 1.2 × 10<sup>-3</sup> g L<sup>-1</sup> for midazolam and no measurable drift of potential readings after successive injections of a 1.0 × 10<sup>-1</sup> g L<sup>-1</sup> solution for a week. The miniaturized electrode kept working continuously for 8 h a day, non-stop, for 1 month. The practical lower limit of detection was of 6.8 × 10<sup>-2</sup> g L<sup>-1</sup> for diazepam selective electrode. Memory effects were observed after successive alternate injections of diazepam

**Table 2b**  
Potentiometric working characteristics for diazepam ion-selective electrodes

Membrane type	II	IV	V
Slope (mV dec <sup>-1</sup> )	80.4 ± 1.7	74.5 ± 1.9	67.6 ± 3.0
LLLR (g L <sup>-1</sup> )	(8.8 ± 1.6) × 10 <sup>-2</sup>	(5.7 ± 0.9) × 10 <sup>-2</sup>	(4.9 ± 1.5) × 10 <sup>-2</sup>
PDL (g L <sup>-1</sup> )	(4.2 ± 0.5) × 10 <sup>-2</sup>	(3.4 ± 0.6) × 10 <sup>-2</sup>	(3.0 ± 0.9) × 10 <sup>-2</sup>
C <sub>max</sub> (g L <sup>-1</sup> )	12.8 × 10 <sup>-1</sup>	7.1 × 10 <sup>-1</sup>	12.8 × 10 <sup>-1</sup>
Response time (s)	<30	<30	<30
pH	[1.9;2.4]	[1.8;2.3]	[1.9;2.7]

**Table 3**  
Potentiometric selectivity coefficient ( $K_{Am^+, Interf^+}^{Pot}$ ) determined according to the matched potential method

Interference	Benzodiazepines/interference	Concentration	
		Midazolam	Diazepam
		$K_{Am^+, Interf^+}^{Pot}$ <sup>a</sup>	
Lactose	1/0.1	0.1	0.4
	1/1	0.01	0.08
	1/10	0.001	0.01
Starch	1/0.1	0.1	0.3
	1/1	0.04	0.2
	1/10	0.01	0.02
Magnesium stearate	1/0.1	0 <sup>a</sup>	0 <sup>a</sup>
	1/1	0 <sup>a</sup>	0 <sup>a</sup>
	1/10	0 <sup>a</sup>	0 <sup>a</sup>
Ammonium chloride	1/1	0	0
	1/10	0.01	0.002
	1/100	0.006	0.004
Sodium chloride	1/1	0	0.02
	1/10	0.008	0.006
	1/100	0.008	0.007

<sup>a</sup> Magnesium stearate is no water soluble.

solutions with very different concentrations, although no measurable drift of potentials after linear curve fitting on the data set collected after successive injections of a  $1.0 \times 10^{-1} \text{ g L}^{-1}$  solution for a week. When coupled to the SI-LOV the electrode kept working continuously for 8 h a day, non-stop, for 15 days.

### 3.3. Selectivity of the miniaturized electrodes

Various authors [21–24] agree that for solid-state membrane electrodes the apparent selectivity coefficients measured under transient flow injection conditions may differ significantly from those measured under batch conditions. The interference process is highly dependent on the rate of diffusion and the exchange reaction of the interfering ion [12]. Under flow conditions the time of interaction with the membrane surface is usually short. Hence, the influence of inorganic species present in pharmaceutical formulations were evaluated as interferences under the described flow system. Interference substances were prepared in the respective solvent used for midazolam and diazepam. The degree of interference was calculated by the MPM [18] (Table 3). Selectivity coefficients, using matched potential method, were defined as the activity ratio of the primary ion and the interfering ion, which gave the same potential change in a reference solution. Coefficients lower than 0.3 were obtained for all species evidencing the absence of significant interferences.

### 3.4. Real sample analysis

The midazolam concentration in Dormicum (labelled with 15 mg/tablet) was assessed during two consecutive days performing in each day one determination at morning and other at the afternoon. The mean result obtained was of 14.75 mg/tablet with an R.S.D. of 1.57% ( $n=4$ ) and a recovery of  $100.7 \pm 1.2\%$ . For comparison purposes the same sample was also assayed using the method proposed by Pfenndt et al. [3], once results provided by the European Pharmacopoeia [1] procedure were very irreproducible. The mean value obtained was of 14.89 mg/tablet with an R.S.D. of 0.6% ( $n=4$ ). A comparative evaluation of the accuracy and precision of both procedures was done using the  $t$ -student test and the Fischer test and compared with the tabulated ones for the level of confidence of 95% (Table 4). The same determination conditions were applied for pharmaceuticals containing diazepam were

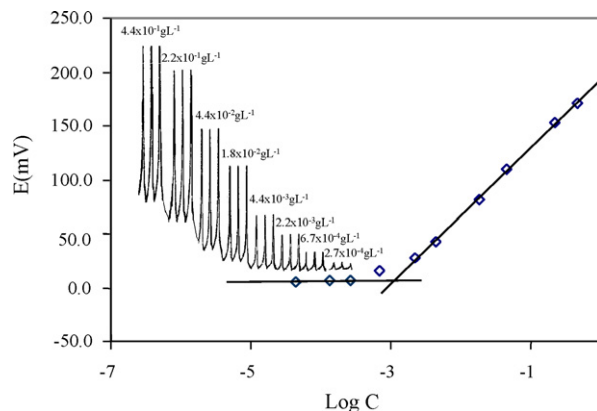
**Table 4**  
Results obtained in the analysis of pharmaceutical formulations available in the Portuguese drug stores by the proposed potentiometric SI-LOV procedure and the chromatographic procedure

Pharmaceutical formulation	Reference method	Proposed method	$F_{Calc}$	$F_{Tab}$	$t_{Calc}$	$t_{Tab}$
<i>Midazolam</i>						
Tablets						
	14.76	14.63	7.12	9.28	1.13	4.30
Dormicum (15 mg)	14.91	14.92				
	14.93	14.96				
	14.95	14.48				
<i>Diazepam</i>						
Capsules						
	3.40	3.82 <sup>a</sup>	4.22	9.55	3.79	4.30
Bialzepam (3 mg)	3.36	3.26				
	3.36	3.30				
	3.37	3.33				
Tablets						
	5.73	5.61	2.20	9.28	1.72	3.18
Unisedil (5 mg)	5.71	5.59				
	6.00	5.72				
	6.01	5.84				
Extended-release capsules						
	11.72	12.37	1.64	9.28	0.43	3.18
Bialzepam Retard (10 mg)	11.70	11.40				
	10.83	10.93				
	10.84	11.10				
Injection						
	5.42	5.32	1.45	9.28	0.61	3.18
Bialzepam (10 mg/2 mL)	5.45	5.32				
	5.29	5.43				
	5.48	5.45				

<sup>a</sup> This value was rejected for the statistical treatment.  $F_{Calc}$ , Fischer test (calculated);  $F_{Tab}$ ,  $F$  tabulated value for 95% confidence level;  $t_{Calc}$ ,  $t$ -student test (calculated);  $t_{Tab}$ ,  $t$ -tabulated value for two-tail 95% confidence level.

tablets (Unisedil–5 mg), capsules (Bialzepam–3 mg, Bialzepam Retard–10 mg) and injectable (Diazepam–5.37 mg mL<sup>-1</sup>) forms are available on the Portuguese market. The obtained recovery percentages were between 97 and 104% for diazepam formulations.

In Fig. 3, a typical calibration recording for midazolam determination is shown as inset, where a shift of the baseline is observed after injection of the more concentrated solutions. In this particular case measurements are performed considering the baseline potential obtained for the carrier solution and the peak potential. In all cases a comparable accuracy and precision was found between the proposed and the chromatographic procedure used for comparison.



**Fig. 3.** Typical recording of a calibration procedure. Inset: calibration curve of midazolam ion-selective electrode based on  $\alpha$ -cyclodextrin, coupled in SI-LOV system.

#### 4. Conclusions

A robust and straightforward automated procedure for the determination of midazolam and diazepam in pharmaceuticals samples is proposed as an alternative to the more tedious albeit generic chromatographic procedures. To achieve this, new midazolam-selective electrodes and diazepam-selective electrodes are proposed, using cyclodextrins as ionophores. Comparing the previous published electrodes with those proposed in this study a much longer useful life were verified without significant variations in their response properties. The general good characteristics, particularly the much improved lifetime led to extend their use as detectors in continuous flow systems. The new potentiometric detector configuration proposed is easy to construct in common laboratories and enables the implementation of low volume detection cell, where the electrical noise, frequently present in more traditional flow potentiometric systems usually requires the resort to an additional grounding electrode.

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