Full Paper

Sequential Injection Lab-on-Valve Procedure for the Determination of Amantadine Using Potentiometric Methods

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Abstract
Amantadine potentiometric detectors were developed, evaluated and incorporated in a SIA-LOV manifold in order to accomplish the control of pharmaceutical formulations and urine. The electrodes incorporate α-cyclodextrin as ionophore, dibutyl phthalate or 2-fluorophenyl 2-nitrophenyl ether as plasticizers and potassium tetrakis[3,5-bis-(trifluoromethyl)phenyl]borate (KTFPB) as cationic additive. The slope increased from 61.2 to 63.8 mV decade^{-1} and the practical limit of detection from 2.6×10^{-6} mol L^{-1} to 2.5×10^{-5} mol L^{-1} when the plasticizer was changed from 2-fluorophenyl 2-nitrophenyl ether to dibutyl phthalate. When incorporated in the flow-manifold the membranes composed by dibutyl phthalate or with 2-fluorophenyl 2-nitrophenyl ether presented slopes and a practical limit of detection of 69.8 mV decade^{-1} and 1.5×10^{-4} mol L^{-1} or 73.7 mV decade^{-1} and 5.4×10^{-5} mol L^{-1}, respectively. The electrode presented stable responses for over a year, and were highly selective concerning the representative species of the two sample matrices assayed as interferents. Comparison of obtained results with those provided by reference methods and recovery assays, revealed adequate accuracy for control assays.

Keywords: Lab-on-valve, Ion selective electrodes, Potentiometry, Amantadine, Cyclodextrin

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1. Introduction

Amantadine (1-adamantanamine hydrochloride) (Fig. 1) is prescribed with distinct indications in therapeutics. It is effective as antiviral activity against influenza A infection albeit its side effects on the central nervous system [1]. Probably due to its antimuscarinic activity and influence on the dopamine release and reuptake balance it reduces symptoms associated with multiple sclerosis and parkinsonism [2]. Amantadine is administered orally, and between 50 and 90% excreted in urine largely unmetabolized through either glomerular filtration and tubular secretion [3]. As in the case of many other pharmaceutical drugs, chemical control of amantadine is based on less specific acid-base titration [4] or resorting to liquid chromatography with fluorimetric detection [5], HPLC [6], or gas chromatography with a flame-ionization detector [7]. However large-scale analysis is impaired by non added-value sample pretreatment involving organic solvent extraction and/or chemical derivatization. To accomplish pharmaceuticals control in a simpler and more economical fashion, potentiometric procedures based on amantadine selective membrane electrodes have been described. The selective membranes were based respectively on the use of the ion-pairs 1-adamantylamine-dipicrylamine or 1-adamantylamine-dinonylnaphthalene sulfonic acid [8], and 1-adamantylamine-tetraphenylborate as ion-exchanger[9]. However, in both works the electrodes presented short lifetimes and the use of inner reference solution electrode configurations coupled to flow-injection manifolds reduced the final manifold robustness. Efforts to improve ion-selective electrodes characteristics have been proposed through the use of different classes of species capable of molecular recognition which include cyclodextrins among others [10]. Cyclodextrins (α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin), are composed of six, seven and eight α-(1,4)-linked glycosyl units, respectively [11], with toroidal three-dimensional configuration. The interior of the toroid forms a hydrophobic cage as a result of the electron rich environment provided largely by the glycosidic oxygen atoms, thus enabling the formation of inclusion complexes with different types of guests. In this work the use of cyclodextrins to develop amantadine-selective electrodes with improved characteristics is evaluated. Furthermore, a previously proposed miniaturized electrode configuration [12] is adopted aiming its coupling to a sequential-injection lab-on-valve system (SI-LOV) in order to allow for the determination of amantadine in pharmaceutical formulations and in urine. The SI-LOV is a programmable liquid flow miniaturization concept based on the integration of the flow detection

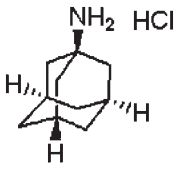

Fig. 1. Chemical structure of Amantadine

cell and other manifold components (connectors, microcolumns, sample flow through port and mixing devices) within a monolithic structure mounted on a multiposition valve [13]. 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 stream selecting valve, into a holding coil [14]. Through down scaling amantadine-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. Electrical noise, that is frequently present in potentiometric based procedures, is significantly reduced.

2. Experimental

2.1. Reagents and Solutions

Distilled, deionized water (conductivity < 0.1 μS cm^{-1}) and analytical grade chemicals were used without further purification, unless otherwise stated. Carboxylated polyvinyl chloride (PVC-COOH), potassium tetrakis[3,5-bis-(trifluoromethyl)phenyl]borate (KTFPB) were from Fluka; tetrahydrofuran (THF) was from Riedel-de-Haën; 2-fluorophenyl 2-nitrophenyl ether (FNDPE), α-cyclodextrin (α-CD), β-cyclodextrin (β-CD) and dibutyl phthalate (DBP) were from Sigma.

A stock solution of amantadine hydrochloride (1-Adamantanamine hydrochloride) (Aldrich) was prepared daily by weighing about 0.1877 g of reagent into a 100 mL volumetric flask and subsequent dilution to the mark with lithium chloride solution with an ionic strength of 0.1 mol L^{-1}. The working calibrating solutions were prepared daily by rigorous dilution with the same ionic strength adjuster. Lithium chloride was also used as carrier in the developed flow system.

Oral pharmaceutical formulation samples were obtained from local pharmacy stores. Sample solutions of commercial amantadine capsules (labeled amount of 100 mg per capsule) were prepared by weighing the content of 20 capsules from the same lot and finely powdering in an agate mortar. Afterwards, an accurately weighed amount of sample, between 35 and 45 mg, was dissolved in 25 mL of LiCl ($I = 0.1$ mol L^{-1}) in order to fit the expected analyte concentration in the linear range of the studied electrode. Urine samples from volunteers were spiked with amantadine hydrochloride solutions ($I = 0.1$ mol L^{-1}).

To perform the reference method proposed by British Pharmacopoeia [4], based in a potentiometric titration, 0.01 mol L^{-1} hydrochloric acid (Fluka), alcohol 96% (v/v) (Merck) and 0.1 mol L^{-1} sodium hydroxide (Sigma) were used.

2.2. Apparatus

A Crison 2002 pH potentiometer (sensitivity: ± 0.1 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 amantadine-selective electrodes. The potentiometric measurements were recorded with a Kipp & Zonen BD 111 recorder coupled to the decimillivoltammeter. The pH values of all solutions and the operational pH range characteristics of the electrodes were determined with a Phillips GAH 110 glass electrode.

The schematic representation of the computer-controlled SI-LOV system used is depicted in Figure 2a. It comprises a Minipuls 3 Gilson (Viliers-le-Bell, France) peristaltic pump with a PVC pumping tube ($\varnothing_{int} = 0.90$ 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 mol L^{-1}), model 6.0727.000 was connected. Four channels ($\varnothing_{int} = 0.5$ 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 and the amantadine selective electrodes top to top (Fig. 2b). A PTFE coil with 60 cm (HC) and flow lines were made with $\varnothing_{int} = 0.5$ 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 potentiometric titration curves for the reference method were obtained using a pH Meter GLP22 from Crison coupled to a glass electrode from Crison n^0 52 – 02.

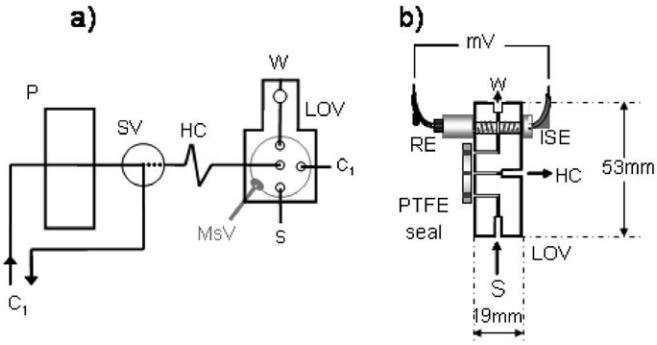

Fig. 2. Schematic view of the proposed set-up. a) The system comprises a peristaltic pump (P) synchronized with a solenoid valve (SV), a holding coil (HC), an acrylic lab-on-valve manifold (LOV) screwed over the rotor of a stream selecting valve (MsV). (W) waste; (C_1) carrier solution and (S) sample. b) Side view of the reference and cyclodextrin electrode screwed on LOV; (RE) reference electrode and (ISE) cyclodextrin-selective electrode.

2.3. Electrodes Membrane Preparation and Electrode Construction

PVC membranes for electrode construction were prepared by mixing 1% (w/w) cyclodextrin and 2% (w/w) additive in 67% (w/w) plasticizer solvent with 30% (w/w) PVC-COOH, that had been previously dissolved in THF. These membranes were dropped directly on the conductive surface of the electrode, made up with a mixture of epoxi resin (Araldite) with graphite powder. Drying was accomplished at room temperature for one day. The electrodes were then soaked in lithium chloride ($I = 0.1$ mol L^{-1}) for 30 minutes before calibration. Miniaturized electrodes, based on the use of commercial end-fittings as electrode body, were developed afterwards accordingly [12] (Fig. 3). The miniaturized electrodes were coupled to the LOV and the conditioning solution was then flowed through at a flow rate of 12.9 μL s^{-1} for the same period of time.

2.4. Procedures

To proceed with the conventional evaluation of the amantadine-selective electrodes, calibration curves were obtained in lithium chloride medium ($I = 0.1$ mol L^{-1}) varying the amantadine concentrations between 1×10^{-6} and 1×10^{-2} mol L^{-1}. The sample pH influence on the electrode response was evaluated for two amantadine solutions (1×10^{-3} and 1×10^{-2} mol L^{-1}) between pH 2 and 11. Therefore, the pH value of 200.0 mL of solution, with adjusted ionic strength ($I = 0.1$ mol L^{-1}) varied according to the addition of NaOH or H_2SO_4 concentrated solutions. Considering electrode evaluation using the SI-LOV system, it should be first stressed that according to previous reports [15], the use of a peristaltic pump to drive precise small fluid volumes requires a three-way solenoid valve activated in a synchronized way relatively to a preset position of the head of the peristaltic pump. The activation of the peristaltic pump at a rotation speed set before each step and the flow rate produced, occurred within a brief yet important interval when compared with the rate needed to drive low volumes. The solution volumes driven to the holding coil also depended on the position of the pump rollers at the beginning of each determination. This way, with a pumping tube of 1.60 mm internal diameter it is possible to drive volumes of solutions higher than 8 μL with a precision above 4%. Thus, 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 190 μL with a settled pumping rate of 8 μL s^{-1}. Then, amantadine hydrochloride solutions at different concentrations were driven at 14 μL s^{-1} during 14 s by the port 2 (sample port in Fig. 2a) and sent towards the flow-through detection cell at a flow rate of 8 μL s^{-1} during 40 s. Then, during 100 s the detection cell is cleaned at a flow rate of 13 μL s^{-1}.

Lithium chloride ($I = 0.1$ mol L^{-1}) was chosen as carrier solution. Interference evaluation presented in the sample matrices (like lactose, starch and magnesium stearate for a commercial amantadine sample, and ammonium chloride, uric acid, urea, sodium chloride and creatinine for human urine) was performed using the matched potential method (MPM) [16] in order to assess the effect of these interferents in the optimized analytical procedure. The selectivity coefficient in this case is defined as the activity (concentration) ratio of the primary ion and the interfering ion which gives the same potential change in a reference solution.

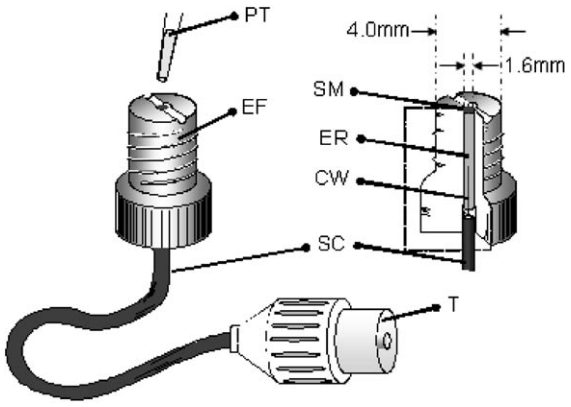

Fig. 3. Schematic view of the miniaturized cyclodextrin-selective electrode obtained by drop wising 20 μL of the sensor solution from a 100 μL pipette tip (PT) over the central hole of a commercial end fitting (EF), previously filled with an conductive epoxi resin (ER) in contact with the central wire (CW) of a shielded cable (SC). (SM) sensing membrane; (T) terminal connector to decimillivoltammeter.

3. Results and Discussion

3.1. Evaluation of Conventionally Shaped Electrodes

Initially, several electrodes based on membranes incorporating β-CD were implemented resorting to plasticizers with increasing dielectric constants, i.e., dibutyl phthalate and 2-fluorophenyl 2-nitrophenyl ether, as well as different cationic additives (KTFPB and KTpClPB). For these electrodes, nernstian slopes were gathered in the first calibration. However, the slopes decreased about 15% for a second calibration procedure and after subsequent ones until no electrode response was obtained. This behavior was interpreted as being related with the high stability constant of the β-CD/amantadine inclusion complex formed [17] which impaired the maintenance of low constant amantadine activity in the membrane phase. For that reason, more attention was given to similar membranes now incorporating α-cyclodextrin. Using the two plasticizers mentioned before, it was observed that both presented constant slightly super-nernstian responses with slopes of 63.8 ± 0.6 mV dec^{-1} for the electrodes based on DBP membranes and

61.2 ± 0.9 mV dec^{-1} for the ones based on FNDPE membranes, respectively (Table 1). The last ones needed more time to achieve constant potentials ($\approx$40 s), but both the lower limit of linear response (*LLLR*) and practical detection limit (*PDL*) values were improved in almost one order of magnitude. When comparing the performance of both kinds of electrodes with those previously proposed [8, 9] better response characteristics were generally obtained. This behavior is in accordance with the general performance improvements observed for other ionophore based electrodes in which the main free ion activity in the membrane phase is more effectively stabilized [18]. Also the useful life of the electrodes was extended. Abdel-Ghani et al. [9] mention for their amantadine electrode a reduction of 4 mV per concentration decade after 24 h and the resort to low temperature conditioning to enhance its usefulness to about one week. Comparatively, the lifetime of the electrodes presented in this work is longer than a year without any particular conditioning procedure. The effect of pH on the potential was also evaluated in the range between pH 2 and 11 for both electrodes (Fig. 4). Between pH 2 and 8.5 the potential variation registered is smaller than 5 mV, independent of the amantadine concentration used in the assays. For pH values higher than 8.5, potential increase and decrease depending on the amantadine concentration in the solution and also on the mediator solvent used in the membrane preparation. The diagrams shape near the pK_a value are correlated to interfering extension of sodium used to perform the experiment and the type of mediator solvent used. This is observed mainly for electrodes containing FNDPE, suggesting a different response mechanism of the membranes, maybe involving the formation of complexes between α-cyclodextrin and amantadine with different stoichiometries.

Table 1. General working characteristics of the conventionally shaped amantadine ion-selective electrodes. *LLLR*: lower limit of linear response; *PDL*: practical detection limit; DBP: dibutyl phthalate; KTFPB: potassium tetrakis[3,5-bis-(trifluoromethyl)phenyl] borate; FNDPE: 2-fluorophenyl 2-nitrophenyl ether; DOP: dioctyl phthalate; *o*-NPOE: 2-nitrophenyl octyl ether; PVC-COOH: poly(vinyl chloride) carboxylated.

Reference	[8]		[9]		Proposed electrode	
Electrode construction	Ion pair complex; stainless steel as internal reference element	Ion pair complex; with inner reference solution	Ion exchanger; coated graphite electrode	Ion exchanger; with inner reference solution	Complexation; coated graphite electrode	
Membrane composition	Graphite impregnated in 1-adamantanamine-dipicrylamine	1-Adamantanamine-dinonylnaphthalene sulfonic acid $+o$-NPOE $+$ PVC	Amantadinium tetraphenylborate $+$ DOP $+$ PVC	Amantadinium tetraphenylborate $+$ DOP $+$ PVC	KTFPB $+$ DBP $+$ α-CD $+$ PVC-COOH (A)	KTFPB $+$ FNDPE $+$ α-CD $+$ PVC-COOH (B)
Slope (mV/dec)	50.4 ± 0.9	55.6 ± 0.8	56.4	56.8 ± 2	63.8 ± 0.6	61.2 ± 0.9
LLLR (mol L^{-1})	5×10^{-4}	1×10^{-5}	2×10^{-4}	2×10^{-5}	4.3×10^{-5}	2.8×10^{-6}
PDL (mol L^{-1})	5×10^{-5}	4×10^{-6}	–	–	2.5×10^{-5}	2.6×10^{-6}
Reproducibility (mV dec^{-1})	–	–	–	–	±1.9	±0.9
Response time (s)	120	30	–	–	<3	>40
pH	[5; 8]	[2; 9]	[2.5; 8.0]	[2.5; 8.0]	[2.0; 8.5]	[2.0; 9.0]
Analytical application	Amantadine-HCl tablets (100 mg)		Adamine capsule (100 mg)		Parkadina capsule (100 mg); Urine	

3.2. Evaluation of the Miniaturized Electrodes Coupled to a SI-LOV System

3.2.1. Optimization of the Flow Parameters

To optimize the flow conditions of the developed SI-LOV setup, different sample volumes were initially driven into the holding coil and then pumped towards the detector. The transient signals obtained increased proportionally to the sample injection volume, becoming independent above 190 μL. The dependence of the peak heights and time to reach the baseline on the flow rate were also optimized. For 40 seconds the sample was pushed to the detector at the flow rate of 8.1 μL s^{-1}. Then, the flow rate of the carrier is raised to 12.9 μL s^{-1} to achieve fast reestablishment of the baseline potential signal (Table 2). The required flow rate for this type of set-up was 13 times smaller than the Abdel-Ghani system (9.7 mL min^{-1}) based on flow injection analysis [9]. General working characteristics of the electrodes were evaluated by making calibration curves in the range between 3×10^{-5} and $1 \times$

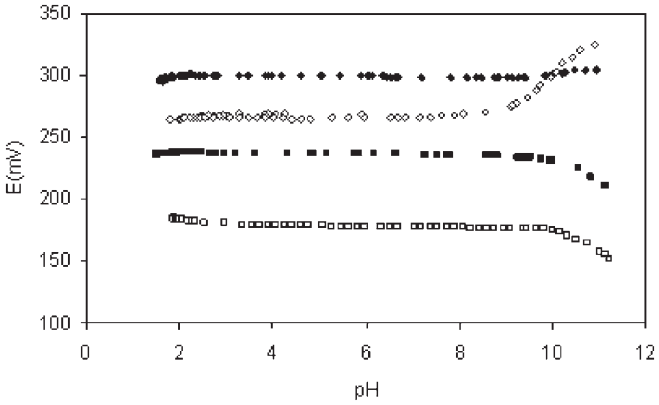

Fig. 4. Effect of pH variation on electrode response for 10^{-2} (◆,◇) and 10^{-3} mol L^{-1} (■,□) amantadine hydrochloride solutions. Full fill symbols: Electrode based on DBP as plasticizer solvent; Empty symbols: Electrode based on FNDPE as plasticizer solvent

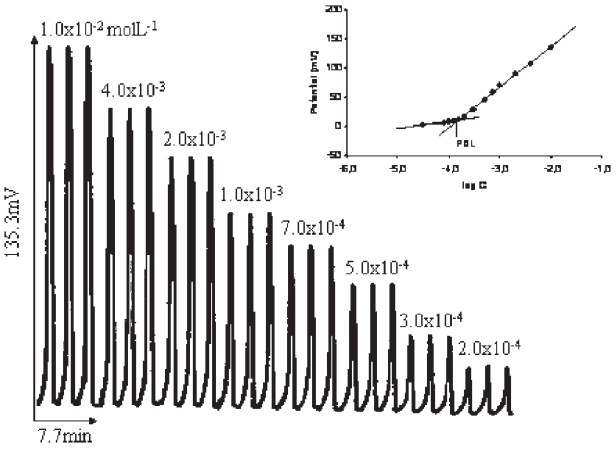

Fig. 5. Typical recording of a calibration procedure. Inset: Calibration curve of amantadine ion-selective electrode based on dibutyl phthalate as plasticizer solvent, coupled in SI-LOV system

10^{-2} mol L^{-1} (Table 3). Super-Nernstian slopes were found both for the electrode containing DBP (69.8 mV dec^{-1}) in the concentration range $2.0 \times 10^{-4} - 1.0 \times 10^{-2}$ mol L^{-1} of amantadine (Fig. 5) and for the electrode containing FNDPE (73.7 mV dec^{-1}), in the range $8.2 \times 10^{-5} - 1.0 \times 10^{-3}$ mol L^{-1} (Table 3). The slope increment found for the studied electrodes when coupled in the SI-LOV system, also reported for periodate electrodes [12] is probably due to the diffusion gradient near the membrane interface in flow conditions. This effect could increase with pressure, which is higher in a LOV-system than in the traditional flow systems. Another important factor is the slow response of the electrode potential to concentration change, especially when low concentrations are measured, which is more pronounced in the FNDPE based electrode. The practical lower limits of detection (*PDL*) were of 1.5×10^{-4} mol L^{-1} and 5.4×10^{-5} mol L^{-1} with relative standard deviations of 0.4% and 4.4%, respectively. In all calibrations, two different solutions (3.8×10^{-4} and 2.1×10^{-3} mol L^{-1}) were processed ten times using the proposed procedure allowing for the calculation of relative standard deviations of 1.7 and 0.1%, respectively. No memory effects were observed after successive alternate injections of different sample amantadine solutions neither measurable drift of potentials after linear curve fitting on the data set collected after successive injections of a 1.9×10^{-4} mol L^{-1} solution for a week. When coupled to the SI-LOV the DBP based electrode kept working continuously for 8 hours a day, nonstop, for 3 months.

Table 2. Optimized flow parameters used in SI-LOV system.

Solution	Time (s)	Flow Rate (μL s^{-1})	Volume (μL)	Direction
Sample	14	13.6	190	Reverse
Carrier	40	8.1	324	Forward
Carrier	100	12.9	1290	Forward

3.2.2. *Selectivity of the Miniaturized Electrodes*

Various authors [19–22] 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 [10]. Under flow conditions the time of interaction with the membrane surface is usually short. Hence, the influence of inorganic species present in pharmaceutical formulations and the main constituents present in collected urine samples were evaluated as interferences under the described flow system. Interference substances were prepared in LiCl 0.1 mol L^{-1}. The degree of interference was calculated by the matched potential method (MPM) [16], two different amantadine hydrochloride concentrations have been found in Parkadina (1.0×10^{-4} and 1.5×10^{-3} g mL^{-1}) and in urine (8.0×10^{-5} and 1.5×10^{-4} g mL^{-1}) used for the assays (Table 4). As interfering species in pharmaceutical formu-

Table 3. Analytical figures of merit of the miniaturized amantadine ion-selective electrodes, coupled in SI-LOV system.

Characteristics in SI-LOV system	Electrode A	Electrode B
Slope (mV $decade^{-1}$)	69.8 ± 0.5	73.7 ± 0.6
Intercept (E_0, mV))	279.3 ± 4.6	324.3 ± 2.0
Practical detection limit (mol L^{-1})	$(1.5 \pm 0.0) \times 10^{-4}$	$(5.4 \pm 0.2) \times 10^{-5}$
Linear range (mol L^{-1})	$2.0 \times 10^{-4} - 1.0 \times 10^{-2}$	$8.2 \times 10^{-5} - 1.0 \times 10^{-3}$

Table 4. Potentiometric selectivity coefficient ($K^{Pot}_{Am+,Interf+}$) determined according to the matched potential method [16].

Parkadina Interferent	Amantadine-HCl : Interferent	Concentration	
		1.0×10^{-4} (g mL^{-1})	1.5×10^{-3}(g mL^{-1})
		$K^{Pot}_{Am+,Interf+}$ [a]	
Lactose	1 : 0.1	9.7×10^{-2}	4.4×10^{-1}
	1 : 1	9.7×10^{-3}	7.7×10^{-2}
	1 : 10	9.7×10^{-4}	1.2×10^{-2}
Starch	1 : 0.1	1.1×10^{-1}	3.0×10^{-1}
	1 : 1	4.3×10^{-2}	1.9×10^{-1}
	1 : 10	1.2×10^{-2}	2.1×10^{-2}
Magnesium stearate	1 : 0.1	0 [a]	0 [a]
	1 : 1	0 [a]	0 [a]
	1 : 10	0 [a]	0 [a]
Urine		8.0×10^{-5} (g mL^{-1})	1.5×10^{-4} (g mL^{-1})
		$K^{Pot}_{Am+,Interf+}$	
Ammonium Chloride	1 : 1	0	0
	1 : 10	9.1×10^{-3}	2.2×10^{-3}
	1 : 100	6.0×10^{-3}	4.1×10^{-3}
Uric Acid	1 : 1	3.2×10^{-2}	4.2×10^{-2}
	1 : 10	6.9×10^{-3}	4.2×10^{-3}
	1 : 100	6.9×10^{-4}	8.3×10^{-4}
Urea	1 : 1	0	0
	1 : 10	0	3.2×10^{-3}
	1 : 100	0	7.3×10^{-4}
Sodium Chloride	1 : 1	0	2.2×10^{-2}
	1 : 10	8.2×10^{-3}	5.7×10^{-3}
	1 : 100	7.5×10^{-3}	6.6×10^{-3}
Creatinine	1 : 1	1.1×10^{-1}	4.2×10^{-2}
	1 : 10	5.0×10^{-2}	4.1×10^{-2}
	1 : 100	3.2×10^{-2}	2.9×10^{-2}

[a] Magnesium stearate is not water soluble.

lations the excipients lactose, starch and magnesium stearate were selected. The main constituents of the urine (ammonium chloride, uric acid, urea, sodium chloride and creatinine) were also studied as interferences. 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.

3.2.3. Real Sample Analysis

The amantadine concentrations in pharmaceutical formulation and in urine were determined using the α-cyclodextrin electrode coupled to the SI-LOV with an optimized procedure. The labeled concentration of amantadine hydrochloride in Parkadina was 100 mg per capsule. To perform the experiments 20 capsules from the same lot was finely powdered in an agate mortar. For 4 days, 3 rigorous amounts of the mixed formulation were solubilized in LiCl 0.1 mol L^{-1}. For Parkadina the obtained results were 108 mg per capsule with an RSD of 2.9% ($n = 12$). For comparison purposes the same sample was also processed using the European Parmacopoeia method [7], based on potentiometric titration and the result was 112 mg per capsule with an RSD of 6.9% ($n = 6$).

Urine samples were spiked with a solution of amantadine hydrochloride 2.00×10^{-3} g mL^{-1} ($I = 0.1$ mol L^{-1}), up to the final concentration of 9.02×10^{-5} g $\cdot$mL^{-1}, in order to represent approximately 10% of metabolization of a typical oral daily intake. The average concentration found was 8.73×10^{-5} g mL^{-1} with an *RSD* of 0.5% ($n = 12$), which represent 96.7% of the added analyte.

4. Conclusions

A robust and straightforward automated procedure for the determination of amantadine in pharmaceuticals as well in urine is proposed as an alternative to the more tedious albeit generic chromatographic procedures. To achieve this, new amantadine-selective electrodes are proposed, using α-cyclodextrin as ionophore. Comparing the previous published electrodes with those proposed in this study a much longer useful life was verified without significant variations in their response properties. Furthermore, the new amantadine potentiometric detector configuration is easy to achieve in common laboratories and allows for the imple-

mentation of low volume detection cell, where the electrical noise, frequently present in potentiometric based procedures usually requires the resort to a grounding electrode.

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