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Comparison of Detectors and Cell Configurations in Flow-Injection Potentiometeric Technique of Pharmaceutical Analysis

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Abstract

Carbon paste and PVC electrodes were constructed and incorporated in the flow injection system for potentiometric determination of amantadine (AM), metformin (MF), ranitidine (RN) and trimetazidine (TRMZ). Wall-jet and continuous flow cells were designed where the wall-jet cell showed better performance regarding the sample dispersion and sensitivity to the change in flow rate. CPEs were more suitable for incorporation in the flow-injection systems due to its fast response, mechanical strength and lifetime. The proposed electrodes showed linear calibration graphs in the concentration range from 10^{-5} to 10^{-1} mol L^{-1} with Nernstian slopes values of 61.4±1.7, 60.1±0.99, 65.5±2.0 and 30.9±1.1 mV $decade^{-1}$ for AM, MF, RN and TRMZ, respectively. The developed electrodes have been successfully applied for flow injection potentiometric determination of the investigated drugs in their pharmaceutical formulation with sample output of 120 samples h^{-1} and the advantage of simplicity, accuracy and automation feasibility.

Key word: Flow-injection potentiometry; CPEs; PVC electrode; Cell configurations; Pharmaceuticals analysis.

1. Introduction

The widespread dosefication and/or adulteration of commercially available pharmaceutical preparations demand reliable method for drug quality control that are preferably selective, rapid and can be undertaken with simple equipment. HPLC procedures [1-2] require sophisticated and expensive instruments, a specific column for each determination and special care with reagents before its injection into the chromatographic system. Furthermore, spectrophotometric and fluorimetric procedures [3, 4] involve long analysis times while NMR spectroscopy procedures are characterized by a narrow calibration range [5]. Polarographic procedures [6, 7] requiring the use of a highly toxic compound like mercury are also found in literature.

In contrast, the electrochemical techniques such as potentiometric methods using ion selective electrodes, can be considered to be advantageous due to their simplicity, short measurement time, low cost, adequate precision and accuracy, wide analytical range (usually more 5 decades) and the ability to measure the activity of various drugs from the formulation matrix in colored or cloudy samples. This makes ISE potentiometry very attractive tools for pharmaceutical analysis [8-12].

Flow injection analysis (FIA) becomes a wide spread methodology characterized by its versatility, ease of automation, high sampling frequency and minimum sample treatment prior to injection into the system. FIA is viewed as a well efficient mean for improving the performance characteristics of ISEs in comparable to the batch measurements as the permanent liquid stream has a conditioning effect on the sensor membrane, leading to a better sensitivity and stability without major errors due to displacement of electrodes between measurements. In addition, the transient nature of the signal in flow injection analysis (FIA) may help to overcome the effect of interfering ions. The remarkable progress in the analytical application of ISEs can be attributed mainly to their widespread use as flow through detectors in automatic analyzers and applications in pharmaceutical analysis [9, 13-16].

The electrochemical properties of sensors incorporated in the detector cell play the predominant role and the most important characteristics of the potentiometric sensors are: measuring range, response time, selectivity, stability and lifetime. For routine analysis, the symmetric configurations (in which the sensing membrane comes in direct contact with two aqueous solutions, the internal with fixed ion activity and the external with the ion activity to be measured) are generally preferred [17-19]. This symmetric configuration still have certain inherent limitations as they are mechanically complicated, bulky, the membrane too fragile for use in flow systems and has shorter lifetime. The constant development of ion selective electrodes is leading to sensors which not only have better performance but are also of simpler and more reliable construction. Solid-contact type electrodes (in which the internal reference element is in direct contact with the electroactive membrane matrix) are popular in flow systems [20-22]. Due mainly to the elimination of the internal reference solution, these electrodes will have certain advantageous such as small size, prolonged lifetime and ability to operate in higher pressure environment where the symmetric ISEs might be damaged. One disturbing drawback of these electrodes is the potential drift depending on the plasticizer content in the membrane matrix, weak adherent between the sensing membrane and the metal support as well as the interaction of the support with the species present in the measuring solution.

Carbon pastes have been employed as useful materials for electrode fabrication since their emergence in the mid 1970s. When compared with PVC membrane sensors, CPEs had the advantages of low Ohmic resistance, short response time in addition to the ease of fabrication and regeneration. Although a considerable attention has been given to the preparation of CPEs, their applications have been focused on preconcentration followed by voltammetric determination of the analyte [23, 24] and just few of these electrodes have been used for FIA potentiometric measurements [25-27].

Amantadine hydrochloride (Am-HCl, tricycle [3.3.1.1] decan-1-amine, hydrochloride) is reported as a synthetic antiviral agent that inhibits the penetration and the replication of virus [28] and had recently used for bird-flow treatment in Egypt. Metformin is used as anti hyperglycemic drugs which management type 2 diabetes [29]. Ranitidine (N-(2-{[5-dimethyl- amino-methyl]-2-furanil}-methyl thioethyl) N-methyl-nitro-1,1- diamino ethane) was extensively used in the treatment of duodenal and gastric ulceration, reflux oesophagitis

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and dyspepsia [30, 31]. Trimetazidine (TRMZ); 1-[(2, 3, 4-trimethox-yphenyl) methyl] piperazine dihydrochloride is a clinically effective antianginal agent that has been used in the prophylaxis and management of angina pectoris, and in ischemia of neurosensorial tissues as in Meniere's disease [32]. Different CPE and PVC electrode were fabricated and optimized for assaying of aforementioned pharmaceutically important drugs under batch potentiometric conditions [33-41].

The aim of the present procedures was to make a comparative assessment of the performance of two cell configurations, namely; wall-jet and continuous flow cells using either CPE or PVC electrodes. The effects of flow rate, sample volume, electrode nature were investigated and optimized to achieve the most favorable analytical characteristics. The developed sensors were applied in the FIA potentiometric determination of AM, MF, RN and TRMZ in their authentic samples pharmaceutical preparations with good accuracy compared with the official methods.

2. Experimental

2.1. Reagents

All reagents were of the analytical grade and bidistilled water was used throughout the experiments. o-Nitrophenyloctylether (o-NPOE, Sigma), PVC (relative high molecular weight, Aldrich) and graphite powder (synthetic 1–2 µm, Aldrich) were used for electrode fabrication. Phosphotungstic acid (PTA, Fluka) and sodium tetrakis (4-fluorophenyl) borate (NaTFPB, Sigma) were used as ion exchangers.

2.2. Authentic samples

Authentic drug samples; amantadine hydrochloride ($C_{10}H_{17}N$. HCl), metformin hydrochloride ($C_4H_{11}N_5$. HCl,), trimetazidine dihydrochloride ($C_{14}H_{22}N_2O_3$. 2HCl) and ranitidine hydrochloride ($C_{13}H_{22}N_4O_3S$. HCl) were kindly provided from National Organization of Drug Control and Research, Giza, Egypt. Stock drug solutions (10^{-1} mol L^{-1}) were prepared by dissolving an appropriate weight of each drug in 100 mL water. The contents of TRMZ, Am, RN and MF were assigned to be 99.5, 99.7, 99.8 and 98.23% using the officinal method [42].

2.3. Pharmaceutical preparations

The pharmaceutical preparation (Adamine capsule, 100 mg amantadine per capsule, Rameda Co. Egypt) was purchased from local drug stores where 8 capsules were weighed and an accurately weighed portion of the powder, equivalent to one capsule was dissolved in 50 mL water. Ten CIDOPHAGE tablets (CID Co. Egypt, either 500 or 850 mg) were weighed and grinded to finely divided powder. An accurate weight of the powder contain 500 mg MF was mixed with 50 mL water, stirred well and filtered into 100 mL volumetric flask. Ranitidol® tablets (El-Nasr Pharmaceutical Chemicals Co., Abu-Zaabal, Egypt, 150 mg) were used as ranitidine samples, the contents of five tablets of the drug formulations were weighed, grinded and amount equivalent to one tablet was dissolved in the minimum volume of water and filtered into a 100 mL calibrated flask. Metacardia® tablets (Global Napi Pharmaceuticals, Egypt, labeled to contain 20 mg of trimetazidine dihydrochloride per tablet) were purchased from local drug stores. Ten tablets were weighed, grinded and dissolved in the minimum volume of bidistilled water and filtered into a 100 mL calibrated flask.

2.4. Apparatus

A schematic diagram of the flow injection manifold (Fig. 1) was composed of four channel peristaltic pump (MCP Ismatec, Zurich, Switzerland) and sample injection valve (ECOM, Ventil C, Czech Republic) with exchangeable sample loops (50-1000 µL). Solutions transferring were Tygon tubes (Cole-Parmer, USA, 95609-48, 2.8 mm i.d.). The flow injection measurements were carried out in a two-line system; the sample was injected into a distilled water stream, which then merged with another stream of distilled water. In both lines, the same tubing size was used, offering the same flow rate. The connector of the two streams was linked to the detector by a 5 cm tube. The change in the electrode potential against Ag/AgCl double-junction reference electrode (Metrohm, Art. no. 6.0726.100) was monitored using 46-Range Digital Multimeter with PC interface.

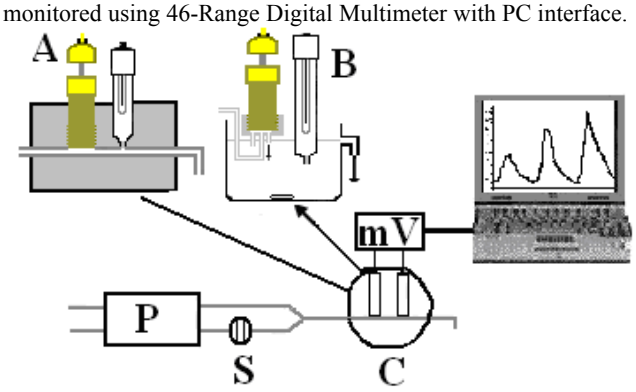

Fig. 1: Scheme of the FIA set-up: peristaltic pump (P); sample injection valve (S); flow cell (C); wall-jet cell (A) and continuous flow cell (B).

In the wall-jet cell, a Perspex cup (internal radii is 3.5 mm) with axially positioned inlet Tygon tubing was fixed with the electrode body. The flow cup with ISEs, reference electrode and the outlet tubes were placed in a beaker, where the level of the solution was kept 1 cm above the electrode surface. The nozzle, which is at the lower side of the cell, is vertically positioned in relation to the electrode surface, from which it is approximately 2 mm away. The Perspex detector cell was designed to allow the change of the distance between the electrode surface and the injection nozzle through an internal screw in the cell body as well as external screw on the electrode which controls the cell dead volume. The stream of the solution wets the whole area of the electrode surface and, pouring down the reference electrode, effectuates the electric contact between the working and reference electrode.

In the continuous flow cell, a Perspex cubic bulk was used. Two cylindrical holes with internal screw were grooved in the Perspex bulk for fixation of both working and reference electrodes. An internal tube within the Perspex was made for connecting the two electrodes and flowing the solutions.

2.5. General Procedures

2.5.1. Sensor construction

The fabrication of both PVC and CPEs was described in details elsewhere [41, 42]. The prepared PVC electrodes were filled with 10^{-2} mol L^{-1} KCl and 10^{-2} mol L^{-1} drug solution using Ag/AgCl as internal reference electrode followed by soaking the electrodes in 10^{-2} mol L^{-1} of drug under the investigation for 24 h. CPEs were prepared by intimate mixing of graphite powder, modifier and pasting liquid in an agate mortar where the result paste was used to fill the electrode body [24] and soaked in 10^{-3} mol L^{-1} of the drug solution. Regeneration of the electrode surface was obtained by screwing the piston and polishing with a very smooth wet filter paper.

2.5.2. Calibration of sensors

200 µL of freshly prepared drug solutions covering the range from 10^{-6} to 10^{-1} mol L^{-1} was injected in the flowing stream (45 mL min^{-1}) and the corresponding peak heights were recorded and used to draw the calibration graphs.

2.5.3. Pharmaceutical preparations

200 µL of different pharmaceutical preparations were injected where the peak heights were measured at the optimum conditions and compared to those obtained from injecting standard solutions of the same concentration.

3. Results and Discussion

The performances of FIA-ISE systems are normally linked to peak height and residence time (time to recover the base line). These two parameters are controlled by the dispersion of the sample, flow cell manifold and the response time of the specific electrode. The flow cell must provide low dead volume, fast response, good wash characteristics, ease of construction, and compatibility with different electrodes designs. Using the Prespix as material for cell manufacturing enables visual observation of any trouble in the solution during measurements.

3.1. Optimization of FIA conditions

3.1.1. Effect of the flow rate

The dependency of the peak heights and residence time on flow rate in both flow cells was studied using the AM-CPE. 200 µL of AM solution (10^{-2} mol L^{-1}) was injected at different flow rates (7-105 mL min^{-1}) for both cells (Fig. 2, 3). The residence time was inversely proportional to the flow rate of the sample at the electrode surface. With the wall-jet flow cell, the residence time decreased from 40 to 11s by increasing the flow rate from 7-105 mL min^{-1} compared with 85 to 18s for continuous flow cell within the same flow rate range.

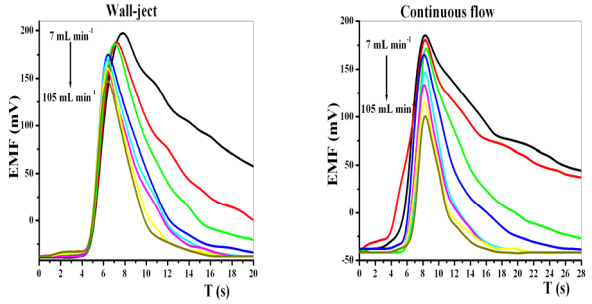

Fig. 2: Effect of the flow rate on the CPE performance using wall-jet and continuous flow cells via injection of 200 µL of 10^{-2} mol L^{-1} AM solution.

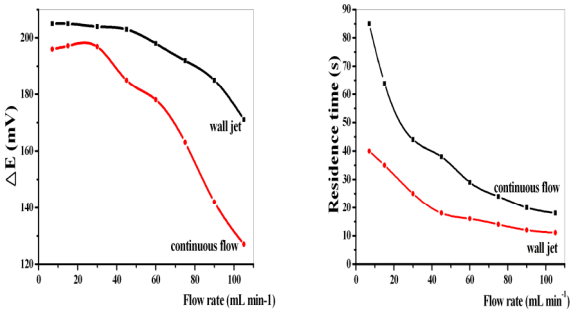

Fig. 3: Effect rate on the peak heights and residence time of CPE performance using wall-jet and continuous flow cells via injection of 200 µL of 10^{-2} mol L^{-1} AM solution.

Using wall-jet cell, the peaks heights of the AM-CPE decreased from 205 to 171 mV by raising the flow rate from 7-105 mL min^{-1}. Contrary, the peak heights with continuous flow cell showed more sensitivity towards the increase in the flow rate as the peak heights decreased to 65% from its original value by changing the flow rate from 7-105 mL min^{-1}. To compromise between the peak heights and residence time, flow rates 45 and 30 mL min^{-1} were selected for wall-jet and continuous flow cells, respectively.

3.1.2. Effect of the sample volume

After setting the optimum flow rates, the injected sample volumes were varied from 50 to 1000 µL. In general, the higher sample volume, the greater the peak heights and residence time. Sample loops of size 200 and 500 µL were selected for the wall-jet and Continuous flow cells, respectively, giving the maximum peak height, less consumption of the reagents, and a shorter time to reach the base line.

3.1.3. Effect of cell design

Theoretically, the wall-jet cell should perform better than the continuous flow cell. In this configuration, the incoming stream of solution passes through the injecting nozzle, hitting the center of the electrode and spreading across its surface (Fig. 1) where the whole sample comes into contact with the sensing surface, ensuring optimum sensitivity. On the other side, with the continuous flow cell, the cell will move inside the internal tubing till reaches the start electrode surface will cause less sensitivity. The superiority of the tubular detector may be attributed to its geometry and the use of a reference electrode close to the sensing membrane which reduces signal noise, stabilizes the baseline, and decreases the drift

The obtained results suggested using of the wall-jet cell as it is less sensitive to the change in the flow rate, less dispersion and consumption of the sample solution. Another advantage of such cell is the ability to control the distance between the electrode surface and the injecting nozzle (through a screw on the electrode Teflon body and the internal surface of the cell). The wall-jet cell size was 77 mm^3 compared with 246 mm^3 for the corresponding continuous flow cell. This arrangement enables the solution to be washed out very quickly from the surface producing higher sampling throughput (120, 70 sample h^{-1} for wall-jet and continuous flow cells, respectively). Under the optimum flow rate, sample volume and injecting 10^{-2} mol L^{-1}, the obtained peaks is relatively higher in case of wall-jet with short residence time (Fig. 2, 3).

In flow injection apparatus, a physical dispersion of the sample within the carrier stream solution occurs, leading to an analytical signal with reduced intensity [43]. Sample dispersion depends largely on the injected volume, flow rates and cell design. Under the above optimum conditions of injection volume (200, 500 µL) and flow rate (45, 30 mL min^{-1}), the dispersion coefficient was found to be 1.18 and 1.23 for wall-jet and continuous flow cells respectively. This limited dispersion aids to reach the optimum sensitivity and fast response of the electrode.

3.1.4. Effect of the electrode type

In flow injection systems combined with ISEs, systems performance and analytical throughput are controlled by the performance of the specific electrode used which depends mainly on the sensitivity and the response time of the electrode used.

Both CPE and PVC electrodes were incorporated in the wall-jet flow cell at the optimum flow rate and injected

sample volume. 200 µL of AM solutions (from 10^{-5} to 10^{-1} mol L^{-1}) were injected in the flow stream and the electrode responses were recorded (Fig. 4).

Incorporation of PVC electrode in the flow system was unsatisfactory due to damage of the sensing membrane during electrode holding in the cell or operation at high flow rate. Contrary, incorporation of CPE in the FIA system was much easier due to the solid nature of the electrode as well as absence of the membrane and internal reference solution. CPEs showed fast response time and stable potential readings as the residence time ranged between 10 and 25s compared with 14-40s for PVC electrode which reflected on the sampling output (120 and 85 sample h^{-1} for CPE and PVC electrodes, respectively). The difference in the electrode performances under FIA condition may be attributed to the difference in the response time of the tested electrodes as CPE is characterized by the fast response (1.6 and 4s for CPE and PVC, respectively [40, 41]).

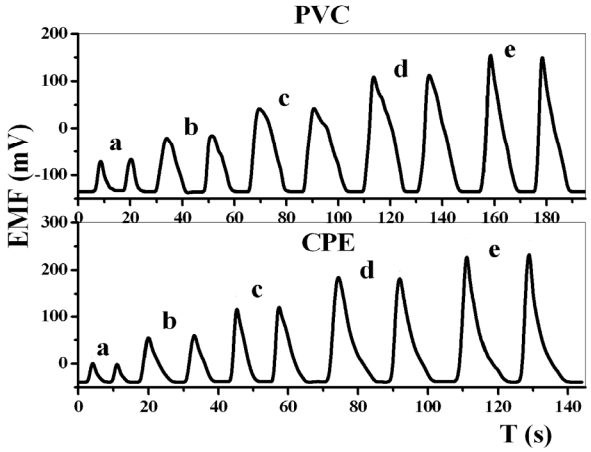

Fig. 4: Flow injection potentiometric determination of AM using PVC and CPE electrodes with wall-jet cell at flow rate 45 mL min^{-1} and injected volume 200 µL: (a) 1×10^{-5}, (b) 1×10^{-4}, (c) 1×10^{-3}, (d) 1×10^{-2} and (e) 1×10^{-1} mol L^{-1} AM.

Generally, the composition of the carrier solution affects the response behavior of ISEs in terms of the base line stability. Our initial experiments showed that the tested electrodes showed stable potential readings so distilled water can be used as a carrier solution. When testing buffers as carrier solution, CPEs showed unstable potential reading.

3.2. Calibration plots

In potentiometric measurements using FIA, the electrode potential depends on the activity of the main ion sensed. The potentiometric response characteristics of drug CPEs sensors were evaluated according to IUPAC recommendations [44]. Linear calibration graphs were obtained by plotting the peak heights versus the logarithmic concentration of each drug. Fig. 5 showed peaks from the proposed electrode system when 200 µL of drugs solutions at various concentrations were injected in the flowing stream (45 mL min^{-1}).

The fabricated electrodes were prepared by incorporating the ion-pairing reagent (either phosphotungstic acid or sodium tetrakis (4-fluorophenyl) borate) within the electrode matrix, followed by soaking the electrodes in the corresponding drug solution. The drug-ion pair formed on the electrode surface will be extracted to the electrode matrix (plasticizer). Such a technique will reduce the time required for the electrode fabrication (there is no need for precipitation and drying of the ion pair) as well as expansion of the application of ion selective electrode for the determination of drugs that cannot be precipitated as a suitable ion pairs. Thus, the proposed electrodes will consequently be incorporated with AM-NaTFPB, MF- NaTFPB, RN- NaTFPB or TRMA-$[NaTFPB]_2$ ion pairs. Such ion pairs will have different lipophilicity and different solubility products affecting on the sensitivirty of the proposed electrodes. In addition, when such electrodes were used in potentiometric determination of the ofermentioned drugs, differet potential reading and strandard elecrode potentials (E^o) were obtained.

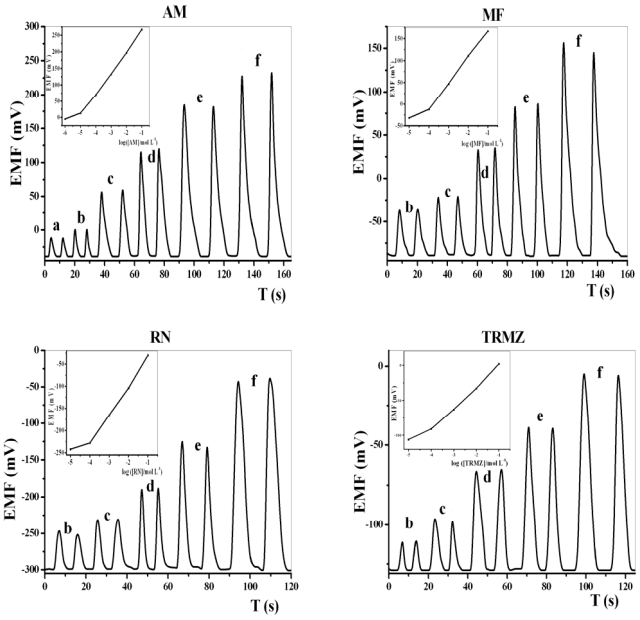

Fig. 5: Flow injection potentiometric determination of AM, MF, RN and TRMZ using CPEs-wall jet system with injection of 200 µL drug solution at flow rate 45 mL min^{-1}: (a) 1×10^{-6}, (b) 1×10^{-5}, (c) 1×10^{-4}, (d) 1×10^{-3}, (e) 1×10^{-2} and (f) 1×10^{-1} mol L^{-1}.

The calibration graphs were linear in the concentration range from 10^{-5} to 10^{-1} mol L^{-1} with Nernstian slopes 61.4±1.7, 60.1±0.99, 65.5±2.0 and 30.9±1.1 mV decade $^{-1}$ for AM, MF, RN and TRMZ, respectively (Table 1). An increase in the slope of the calibration plots was observed compared with batch measurements, where potential is measured under conditions very close to the equilibrium at the membrane solution interface.

The repeatability of the electrode responses was excellent; variation of the peak heights for 10 injections of 10^{-2} mol L^{-1} drug solutions were less than 1mV indicating the high reproducibility of the measurement.

The lifetimes of the sensors were studied by continuously pumping and repeatedly injection of 10^{-2} mol L^{-1} of drug standard solutions at every h. The tested CPEs showed relatively longer lifetime than PVC as the useful operation time was 14 days of continuous measurements.

3.3. Analytical applications

The proposed electrodes were successfully employed for the assay of the tested drugs in their authentic samples as well as pharmaceutical formulations under FIA conditions. The results clearly indicated satisfactory agreement between the drug contents in different samples determined by the developed sensor and official method (Table 2). The time required for sample analysis time was short in case of FIA (about 1min) compared with about 10 min for the standard and potentiometric

titration methods. In FIA measurements, the sample remains in contact with the electrode for a short period of time, the apparent selectivity is expected to be improved compared with batch conditions. Under FIA conditions, the values of selectivity coefficients were calculated based on potential values corresponding to the peak heights for the same concentrations of the drug and the interferents according to the separate solution method (SSM). The prepared sensors exhibit a high selectivity toward tested drugs rather than other pharmaceutical diluents commonly used in drug formulations (e.g., glucose, lactose, starch and mannitol). The selectivity coefficients obtained by the sensors typically agreed with that obtained within references 33-42.

Table 2: Flow injection potentiometric determination of amantadine, metformin, ranitidine and trimetazidine in authentic samples and pharmaceutical preparations

Drug	Sample	Injected (mg)	Recovery (%)*	R.S.D.
AM	Authentic	0.030	103.3	2.2
		0.300	99.4	1.9
		3.000	99.0	1.4
	Adamine 100 mg	0.030	106.6	2.9
		0.320	99.7	2.3
MF	Authentic	0.026	96.1	1.9
		0.260	98.5	1.3
		2.600	99.5	0.9
	CIDOPHAGE	0.079	96.2	3.2
		0.790	97.2	2.9
RN	Authentic	0.063	98.4	2.1
		0.630	102.1	1.7
		6.300	99.7	1.5
	Ranitidine 100mg	0.027	103.7	2.5
		0.270	99.3	2.3
TRMZ	Authentic	0.054	96.3	1.7
		0.540	101.1	1.1
		5.400	102.3	1.0
	Metacardia 20mg	0.032	104.2	2.1
		0.320	102.8	2.0

- Mean recovery and standard deviations for five injection

4. CONCLUSION

The present work demonstrates the incorporation of CPE and PVC electrodes in flow injection determination of amantadine (AM), metformin (MF), ranitidine (RN) and trimetazidine (TRMZ). The home-made flow cell used in the present study allowed operation at higher flow rates with low dispersion factor. CPEs were more suitable for use in flow injection systems due their fast response time and mechanical strength. The proposed electrodes showed Nernstian slopes 61.38±1.72, 60.06±0.99, 65.5±2.03 and 30.9±1.12 mV decade^{-1}for AM, MF, RN and TRMZ, respectively and long operational lifetime. The fabricated electrodes were successfully applied for the potentiometric determination of aforementioned drugs in pure and pharmaceutical forms under FIA conditions with average recoveries comparable to the official methods. FIA allows high sampling output (120 samples h^{-1}) with the possibility for incorporation in routine analysis for drug quality control.

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