http://dx.doi.org/10.5935/0103-5053.20130069

J. Braz. Chem. Soc., Vol. 24, No. 4, 585-594, 2013.
Printed in Brazil - ©2013 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Plastic Membrane, Carbon Paste and Multiwalled Carbon Nanotube Composite Coated Copper Wire Sensors for Determination of Oxeladin Citrate Using Batch and Flow Injection Techniques

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A fabricação e as características de desempenho de três novos sensores potenciométricos para a determinação de citrato de oxeladina são descritas. Os sensores propostos incluem um sensor do tipo membrana em plástico PVC, um sensor de pasta de carbono e um sensor de fio de cobre revestido com compósito de nanotubos de carbono de parede múltiplas (MWCNT). Os sensores são baseados no íon associado oxeladina-fosfotungstato como material eletroativo e dibutilftalato como mediador solvente. Os sensores desenvolvidos exibiram coeficientes quase nernstianos de 58,00 ± 0,22, 55,00 ± 0,69 e 57,80 ± 0,13 mV concentração década^{-1} a 25 ^{o}C, nos intervalos de concentração de citrato de oxeladina de $5,96 \times 10^{-6}$ - $1,00 \times 10^{-2}$, $1,96 \times 10^{-5}$ - $1,00 \times 10^{-2}$ e $3,98 \times 10^{-6}$ - $1,00 \times 10^{-2}$ mol L^{-1} com limites de detecção de $4,14 \times 10^{-6}$, $8,58 \times 10^{-6}$ e $2,51 \times 10^{-6}$ mol L^{-1} de citrato de oxeladina para os sensores de membrana plástica, de pasta de carbono e de fio de cobre revestido com compósito de MWCNT, respectivamente. Os sensores propostos exibiram boa seletividade para oxeladina com relação a um grande número de cátions inorgânicos, açúcares, aminoácidos e vitaminas. Os sensores desenvolvidos foram aplicados com êxito na determinação potenciométrica de citrato de oxeladina em preparação farmacêutica (cápsulas de Paxeladine®) sob condições de injeção em fluxo e batelada. Os sensores também foram aplicados na determinação de citrato de oxeladina em amostras de urina humana utilizando o método de adição padrão.

The fabrication and performance characteristics of three novel potentiometric sensors for the determination of oxeladin citrate are described. The proposed sensors include a PVC plastic membrane sensor, a carbon paste sensor and a multiwalled carbon nanotube (MWCNT) composite coated copper wire sensor. The sensors are based on the oxeladin-phosphotungstate ion associate as electroactive material and dibutyl phthalate as solvent mediator. The developed sensors exhibited near nernstian slopes of 58.00 ± 0.22, 55.00 ± 0.69 and 57.8 ± 0.13 mV concentration decade^{-1} at 25 ^{o}C, in the concentration ranges 5.96×10^{-6} - 1.00×10^{-2}, 1.96×10^{-5} - 1.00×10^{-2} and 3.98×10^{-6} - 1.00×10^{-2} mol L^{-1} oxeladin citrate with limits of detection of 4.14×10^{-6}, 8.58×10^{-6} and 2.51×10^{-6} mol L^{-1} oxeladin citrate for plastic membrane, carbon paste and MWCNT composite coated copper wire sensors, respectively. The proposed sensors exhibit good selectivity for oxeladin with respect to a large number of inorganic cations, sugars, amino acids and vitamins. The developed sensors were successfully applied for the potentiometric determination of oxeladin citrate in the pharmaceutical preparation (Paxeladine® capsules) in batch and flow injection conditions. The sensors were also applied for the determination of oxeladin citrate in human urine samples by using the standard addition method.

Keywords: oxeladin citrate, potentiometry, flow injection, pharmaceutical formulation, human urine

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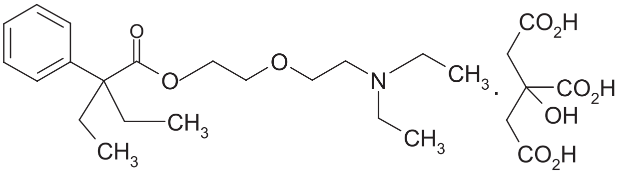
Introduction

Oxeladin citrate, 2-(2-diethylaminoethoxy)ethyl 2-ethyl-2-phenylbutyrate dihydrogen citrate, CAS No. 52432-72-1 (Scheme 1), is a white or almost white crystalline powder soluble in water that exhibits polymorphism. It is given orally as a centrally acting cough suppressant for non-productive cough.[1,2] It acts as an antitussive devoid of opioid side effects.[3]



Scheme 1. Structural formula of oxeladin citrate.

The literature survey reveals that the number of the analytical methods referring to the drug is relatively limited. The reported methods include spectrophotometry,[4-6] gas chromatography-mass spectrometry (GC-MS)[7] and high performance liquid chromatography (HPLC).[8] The drug in the pure form is determined by the British Pharmacopoeia official method,[2] which involves titration of the drug by perchloric acid in anhydrous acetic acid, and determination of the end point by potentiometric method. No potentiometric sensors for the determination of this drug have been reported in the literature to date. Potentiometric methods with ion selective electrodes have proved to be effective for the analysis of pharmaceutical compounds and biological samples because these sensors offer the advantages of simple design, reasonable selectivity, fast response time, applicability to colored and turbid solutions and possible interfacing with automated and computerized systems.[9] Carbon nanotubes are widely used in several fields in material science. It is a major component in nanotechnology.[10,11] The recent discovery of carbon nanotubes (CNTs) attracted much attention because of their dimensions and structural sensitive properties.[12] The nanotubes consisted of up to several tens of graphite shells (so called multiwalled carbon nanotubes, MWCNT) with adjacent shell separation of ca. 0.34 nm, diameters of ca. 1 nm and high length/diameter ratio. Carbon nanotubes have a novel structure, narrow distribution size, high surface area, high conductivity, remarkable mechanical properties and high stability. The introduction of CNTs into a polymer matrix improves the electric conductivity and the mechanical properties of the polymer matrix.[10] In the present work, a plastic membrane sensor, carbon paste sensor and MWCNT composite coated copper wire sensor for oxeladin cation were constructed and the performance characteristics were studied. The proposed sensors were used to determine oxeladin citrate in bulk, pharmaceutical formulation (Paxeladine® capsules) and human urine samples.

Experimental

Reagents and materials

All chemicals were of analytical grade. Double distilled water was used throughout all experiments. Pure grade oxeladin citrate and the pharmaceutical formulation (Paxeladine®, 40 mg capsules) were provided by The Arab Drug Company, Cairo, Egypt. Phosphotungstic acid (PTA), dioctyl sebacate (DOS), 2-nitrophenyl octyl ether (2-NPOE) and tricresyl phosphate (TCP) were from Fluka, tetrahydrofuran (THF), dibutyl phthalate (DBP) and dioctyl phthalate (DOP) from Merck. Graphite powder (1-2 micron) and polyvinyl chloride (PVC) of relatively high molecular weight were from Aldrich. MWCNT, purity more than 95%, were provided from Nanotechnology Unit, Faculty of Science, Beni Suef University, Beni Suef, Egypt.

Apparatus

Potentiometric and pH measurements were carried out using a Seibold G-103 digital pH/mV-meter (Vienna, Austria). A Techne circulator thermostat model C-100 was used to control the temperature of the test solutions. A saturated calomel electrode (SCE) was used as the external reference, while an Ag/AgCl wire was used as the internal reference electrode.

The flow injection (FI) setup is identical to that reported in previous research.[13,14] Figure 1 represents the schematic diagram of the flow injection system used in the measurements.

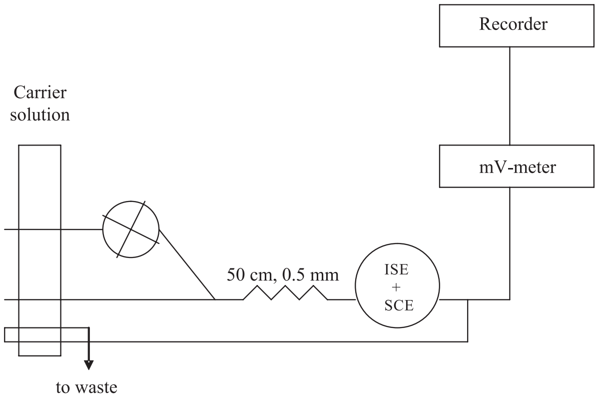


Figure 1. Schematic diagram of the flow injection system used in the measurements. ISE: Ion selective electrode.

Preparation of oxeladin-PT ion associate

The oxeladin-PT ion associate was prepared by mixing 300 mL 10^{-2} mol L^{-1} oxeladin citrate with 100 mL 10^{-2} mol L^{-1} PTA solutions. The obtained precipitate was filtered, washed thoroughly with bidistilled water and dried at room temperature. The composition of the ion associate was found to be 3:1 as confirmed by elemental analysis data obtained at the Microanalytical Research Laboratory in the Cairo University, Giza, Egypt. The percentage values found are 18.40, 3.91 and 0.95 and the calculated values are 18.56, 3.82 and 1.08 for C, H and N, respectively.

Electrodes assembly

Preparation of plastic membrane electrodes

The membrane composition was studied by varying the percentages (m/m) of the ion associate, PVC and DBP, until the optimum composition that exhibits the best performance characteristics were obtained. The membranes were prepared by dissolving the required amount of the ion associate, PVC and DBP in about 6 mL of THF. Four membrane compositions were prepared, containing the oxeladin-PT ion associate in the ratios: 1% (3.5 mg ion associate, and 173.25 mg of each of PVC or DBP), 3% (10.5 mg ion associate, and 169.75 mg of each of PVC or DBP), 5% (17.5 mg ion associate, and 166.25 mg of each of PVC or DBP) and 7% (24.5 mg ion associate, and 162.75 mg of each of PVC or DBP). This cocktail was poured into a 6 cm Petri dish and left to dry in air. The thickness of the membrane was about 0.2 mm. Disks of the membrane were used to assemble the electrodes by the general procedure previously described.[15] The electrochemical system of the conventional plastic membrane electrode is represented as follows: Ag/AgCl/filling solution (1×10^{-1} mol L^{-1} NaCl and 1×10^{-2} mol L^{-1} oxeladin citrate)/membrane/test solution// KCl salt bridge//saturated calomel electrode.

Preparation of carbon paste electrode

The carbon paste was prepared by mixing the required amount of the ion associate, graphite powder and dibutyl phthalate as a pasting liquid (ratio of graphite powder to pasting liquid was 1:1) in a mortar until it was uniformly wetted. Four compositions from the carbon paste were prepared, containing the oxeladin-PT ion associate in the ratios: 1% (3.5 mg ion associate, and 173.25 mg of each of graphite or DBP), 3% (10.5 mg ion associate, and 169.75 mg of each of graphite or DBP), 5% (17.5 mg ion associate, and 166.25 mg of each of graphite or DBP) and 7% (24.5 mg ion associate, and 162.75 mg of each of graphite or DBP). The carbon paste electrode was prepared by successive packing of the carbon paste into the tip end of home made Teflon holder (2 mm) and electrical contact was achieved by stainless steel rod (2 mm) connecting the paste to the mV-meter. The electrochemical system of carbon paste sensor is represented as follows: carbon paste electrode/test solution//KCl salt bridge//saturated calomel electrode.

Preparation of MWCNT composite coated copper wire electrode

Pure copper wire of 2 mm diameter and of 12 cm in length was insulated by tight polyethylene tube leaving 2 cm at one end for coating and 1 cm at the other end for connection. The polished electrode surface was coated with the active membrane by quickly dipping the exposed end 6-8 times into the coating solution prepared by dissolving optimum ion associate composition previously described under plastic membrane composition, 5% ion associate (17.5 mg), 1% MWCNT (3.5 mg), 47% PVC (164.5 mg) and 47% DBP (164.5 mg), and allowing the film left on the wire to dry in air for about 1 min each time until a plastic film of approximately 1.0 mm thickness was formed. The prepared electrode was preconditioned by soaking for 15 min in 1×10^{-3} mol L^{-1} drug solution. The electrochemical system of MWCNT composite coated copper wire electrode may be represented as follows: copper wire/MWCNT composite membrane/test solution// KCl salt bridge//saturated calomel electrode.

Selectivity of the electrodes

The selectivity coefficients ($K^{pot}_{Oxeladin, J^{z+}}$) of the electrodes towards different cationic species (J^{z+}) were determined by a separate solution method,[16] in which the Nicolsky Eisenman equation was used:

$$\log K^{pot}_{Oxeladin}, J^{z+} = \frac{(E_2 - E_1)}{S} + \log[\text{Oxeladin}] - \log[J^{z+}]^{\frac{1}{z}} \quad (1)$$

where E_1 and E_2 are the electrode potentials in 1×10^{-3} mol L^{-1} oxeladin citrate and interfering ions (J^{z+}), respectively, and S is the slope in mV. In the case of ions without charges, the selectivity coefficients were determined by the matched potential method (MPM).[17] In this method, a known activity of oxeladin ion solution is added into a reference solution containing a fixed activity of oxeladin ion $\alpha_{Oxeladin}$, ($\alpha'_{Oxeladin} - \alpha_{Oxeladin}$ is the change in activity), and the corresponding potential change (ΔE) is recorded. Then, a solution of the interfering ion is added to the reference solution until the same potential change (ΔE) is reached. The change in potential produced at the constant background of the primary ion must be the same in both cases

$$K^{\text{pot}}_{\text{Oxeladin},J^{z+}} = \frac{\alpha'_{\text{Oxeladin}} - \alpha_{\text{Oxeladin}}}{\alpha_j} \quad (2)$$

where α_j is the activity of the added interferent.

Potentiometric determination of oxeladin citrate

Oxeladin citrate was potentiometrically determined using the investigated electrodes by the standard addition method.[18]

Determination of oxeladin citrate in the pharmaceutical preparation Paxeladine® capsules

The contents of twenty capsules were accurately weighed and powdered in a mortar. The required amount of powdered capsules to prepare 10^{-3} mol L^{-1} oxeladin citrate solution was dissolved in about 30 mL of bidistilled water and filtered in a 100 mL measuring flask. The residue was washed three times with bidistilled water and the volume was completed to the mark by the same solvent. 50 mL solutions having different concentrations (1×10^{-5} - 5×10^{-4} mol L^{-1} oxeladin citrate) were prepared by accurate dilution. The prepared solutions were transferred into a 100 mL titration cell and subjected to potentiometric determination of oxeladin citrate using standard addition method.

In FI, a series of solutions of different concentrations of Paxeladine® capsules was prepared, and the peak heights were measured, and then compared with those obtained from injecting a standard solution of the same concentration prepared from pure oxeladin citrate.

Determination of oxeladin citrate in spiked human urine samples

Different amounts of oxeladin citrate and 5 mL of human urine from a healthy person were transferred to 50 mL measuring flask and completed to the mark by bidistilled water. The contents of the measuring flask were transferred to a 100 mL titration cell and subjected to potentiometric determination of oxeladin citrate using standard addition method.

Results and Discussion

Optimization of the electrodes in batch conditions

Plastic membrane composition

Four membrane compositions were prepared containing the oxeladin-PT ion associate in the ratios 1, 3, 5 and 7%, and the ratio of PVC to liquid mediator was 1:1. The results indicated that the electrode made by membrane with 5% oxeladin-PT ion associate exhibits the best performance characteristics (slope 58.20 ± 0.22 mV concentration decade^{-1}, linear range 5.96×10^{-6} - 1.00×10^{-2} mol L^{-1} and limit of detection[19,20] 4.14×10^{-6} mol L^{-1}, Tables 1 and 2). The PVC acts as a regular support matrix for the plastic membrane ion selective electrode, but its use requires a plasticizer which acts as a fluidizer allowing homogeneous dissolution and diffusional mobility of the electroactive complex inside the membrane.[21] Five plasticizers, DBP, DOP, DOS, TCP and 2-NPOE, were tested. The results indicated that DBP is the best tested plasticizer. Poor sensitivities for electrodes plasticized by the other plasticizers are due to low distributions of the electroactive complex oxeladin-PT in these solvents.

Carbon paste electrode composition

Four electrodes were prepared containing the oxeladin-PT ion associate in the ratios, 1, 3, 5 and 7% and the ratio of graphite to liquid mediator is 1:1. The results show that the carbon paste electrode with ion associate ratio of 5% has the best performance characteristics, (slope 55.00 ± 0.69 mV concentration decade^{-1}, linear range 5.96×10^{-6} - 1×10^{-2} mol L^{-1} and limit of detection 4.14×10^{-6} mol L^{-1}, Tables 1 and 2). The influence of solvent mediator on the performance characteristics of the

Table 1. Compositions of oxeladin electrodes and the slopes of the calibration graphs at 25 ± 1 °C

Plastic membrane composition / %, m/m					Carbon paste composition / %, m/m				
Ion associate	PVC	DBP	Slope / (mV decade^{-1})	SD / (mV decade^{-1})	Ion associate	Graphite	DBP	Slope / (mV decade^{-1})	SD / (mV decade^{-1})
1	49.5	49.5	54.8	1.00	1	49.5	49.5	51.4	1.01
3	48.5	48.5	57.0	0.39	3	48.5	48.5	54.0	0.28
5	47.5	47.5	58.2	0.22	5	47.5	47.5	55.0	0.69
7	46.5	46.5	57.8	0.22	7	46.5	46.5	54.8	0.26

DBP: dibutyl phthalate; SD: standard deviation.

Table 2. Effect of plasticizers on oxeladin responsive electrodes and the slopes of the calibration graphs at 25 ± 1 °C

Plasticizer	Slope / (mV decade^{-1})	Usable concentration range / (mol L^{-1})	Limit of detection / (mol L^{-1})	SD / (mV decade^{-1})
Plastic membrane				
DBP	58.2	5.96×10^{-6} - 1.00×10^{-2}	4.14×10^{-6}	0.22
DOP	48.9	5.96×10^{-6} - 1.00×10^{-2}	4.14×10^{-6}	2.87
DOS	57.3	5.96×10^{-6} - 1.00×10^{-2}	4.64×10^{-6}	0.71
TCP	53.8	5.96×10^{-6} - 1.00×10^{-2}	4.64×10^{-6}	1.31
2-NPOE	57.5	5.96×10^{-6} - 1.00×10^{-2}	4.47×10^{-6}	0.85
Carbon paste				
DBP	55.0	1.96×10^{-5} - 1.00×10^{-2}	8.58×10^{-6}	0.69
DOP	38.4	1.96×10^{-5} - 1.00×10^{-2}	3.98×10^{-6}	0.87
DOS	25.4	6.31×10^{-6} - 9.12×10^{-5}	5.01×10^{-6}	1.67
TCP	51.9	3.84×10^{-5} - 1.00×10^{-2}	1.21×10^{-5}	1.29
2-NPOE	55.0	3.84×10^{-5} - 1.00×10^{-2}	1.39×10^{-5}	0.21

DBP: dibutyl phthalate; DOP: dioctyl phthalate; TCP: tricresyl phosphate; DOS: dioctyl sebacate; 2-NPOE: 2-nitrophenyl octyl ether; SD: standard deviation.

carbon paste electrode was investigated by using the five plasticizers used in plastic membrane. The results indicated that DBP is the best tested plasticizer.

By using the optimum ion associate composition 5% oxeladin-PT described previously under plastic membrane composition, and using this composition for preparing copper coated wire electrodes, the performance characteristics of the prepared copper coated electrodes were slope 57.5 ± 0.37 mV concentration decade^{-1} at 25 °C, usable concentration range 1.00×10^{-5} - 1.00×10^{-2} mol L^{-1}, limit of detection 4.30×10^{-6} mol L^{-1} and response time ≤ 15 s. By modifying the coating cocktail of the prepared copper coated electrodes by addition of 1% MWCNT, the composition of the prepared MWCNT composite coated copper wire electrode was 5% ion associate, 1% MWCNT, 47% PVC and 47% DBP, it was found a significant improvement of the performance characteristics of the MWCNT composite coated copper electrodes. The characteristics are slope 57.8 ± 0.13 mV concentration decade^{-1} at 25 °C, usable concentration range 3.98×10^{-6} - 1×10^{-2} mol L^{-1} oxeladin citrate, limit of detection 2.51×10^{-6} mol L^{-1} and response time ≤ 5 s.

The potential response characteristics of the proposed electrodes were evaluated according to IUPAC recommendations (Table 3).[22]

The lowering in limit of detection and response time in the case of MWCNT composite coated copper wire electrode may be due to the improvement of electrical conductivity of the PVC matrix by addition of multiwalled carbon nanotubes to this matrix.

Influence of pH and effect of electrolytes

The effect of the solution pH on the electrode response was checked for two concentration of oxeladin citrate (1×10^{-3} and 1×10^{-4} mol L^{-1}) by following the variation in potential with change in pH by addition of very small volumes of hydrochloric acid and sodium hydroxide (each 0.1-1.0 mol L^{-1}). The results indicate that the investigated electrodes showed no pH response over the range 2.0-8.0 and 1.9-6.6 for the plastic membrane and carbon paste electrodes, respectively. Figure 2 represents potential *vs.* pH profiles for the plastic membrane electrode. At higher pH values, the potential decreased due to the gradual increase in the concentration of the unprotonated

Table 3. Response characteristics of oxeladin sensors

Parameter	Plastic membrane electrode	Carbon paste electrode	MWCNT composite coated electrode
Slope / (mV decade^{-1})	58.2	55.0	57.8
Intercept / mV	148.24	312.89	314.85
Linear range / (mol L^{-1})	5.96×10^{-6} - 1×10^{-2}	1.96×10^{-5} - 1×10^{-2}	3.98×10^{-6} - 1×10^{-2}
Limit of detection / (mol L^{-1})	4.14×10^{-6}	8.58×10^{-6}	2.51×10^{-6}
Working pH range	2.0-8.0	2.0-8.0	1.9-6.6
Response time / s	< 10 s	< 10 s	< 5 s

oxeladin resulting in the precipitation of oxeladin base. The electrode response was checked with bidistilled water, 0.04 mol L^{-1} Britton Robinson buffer pH 5.0, 0.1 mol L^{-1} acetate buffer pH 5.0 or 0.1 mol L^{-1} phthalate buffer pH 5.0. The best results were achieved in 0.1 mol L^{-1} acetate buffer in the case of the plastic membrane electrode, but in the case of the carbon paste electrode using bidistilled water, it provided not only a higher nernstian slope but also a stable potential reading. Therefore, acetate buffer was used in the case of plastic membrane electrodes and bidistilled water was used in the case of carbon paste electrodes.

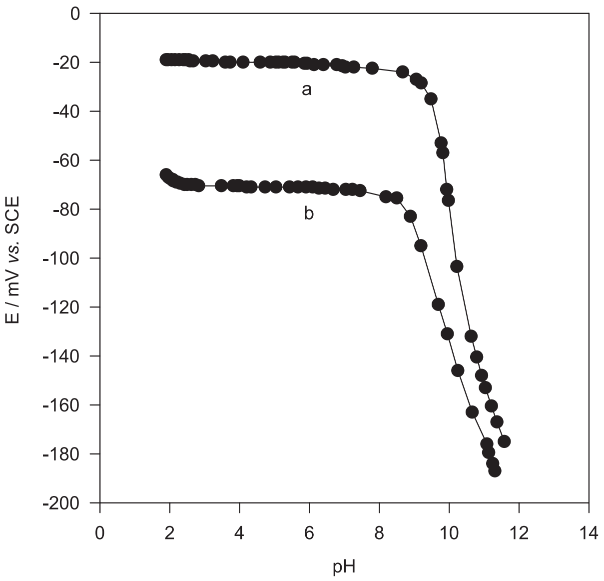


Figure 2. Effect of the pH of the test solution containing 1 × 10^{-3} (a) and 1 × 10^{-4} mol L^{-1} (b) oxeladin citrate on the response of the plastic membrane oxeladin electrode.

The effect of ionic strength on the response of the electrodes was also studied, by measuring the potential values of the electrodes at different electrolyte concentrations, 0.01-1.0 mol L^{-1} NaCl in 0.1 mol L^{-1} acetate buffer pH 5.0 in the case of plastic membrane electrode, and in bidistilled water in the case of carbon paste electrode. The results indicate that there is no effect of ionic strength on the response of these electrodes.

Life time and response time of the sensors

The life time in the case of plastic membrane and MWCNT modified coated copper wire sensors was investigated by performing the calibration graphs periodically and calculating the response slope after these sensors were soaked continuously in 10^{-3} mol L^{-1} oxeladin citrate. The results indicate that in the case of plastic membrane sensor, the slope remains constant near 58.2 mV decade^{-1} for 1 day, then it slightly decreased reaching 57.3, 56.0, 54.1, 51.2 and 46.0 mV decade^{-1} after 3, 5, 7, 14 and 16 days soaking, respectively. In the case of MWCNT modified coated copper wire sensor, the slope remains constant 58.0 mV decade^{-1} for 7 days, then decreases reaching 55.0, 53.0 and 49.0 mV decade^{-1} after 9, 11 and 13 days, respectively.

The life span of carbon paste sensor was investigated by performing the calibration graphs periodically and calculating the response slope after the sensor was left in air without soaking in the drug, the sensor was preconditioned by soaking in 1 × 10^{-3} mol L^{-1} oxeladin citrate for 30 min before use. The results indicate that the slope remains constant near 55.0 mV decade^{-1} for 1 day, and then it slightly decreased reaching about 53.0 mV decade^{-1} after 3 months.

The response time of the proposed sensors was measured after successive immersion of the sensor in a series of drug solutions, each having a 10-fold increase in concentration (1 × 10^{-5} - 1 × 10^{-2} mol L^{-1}). The response time was fast, being nearly instantaneous (5-10 s). A typical potential-time plot for the response of the electrode is shown in Figure 3 as representative figure for the plastic membrane sensor.

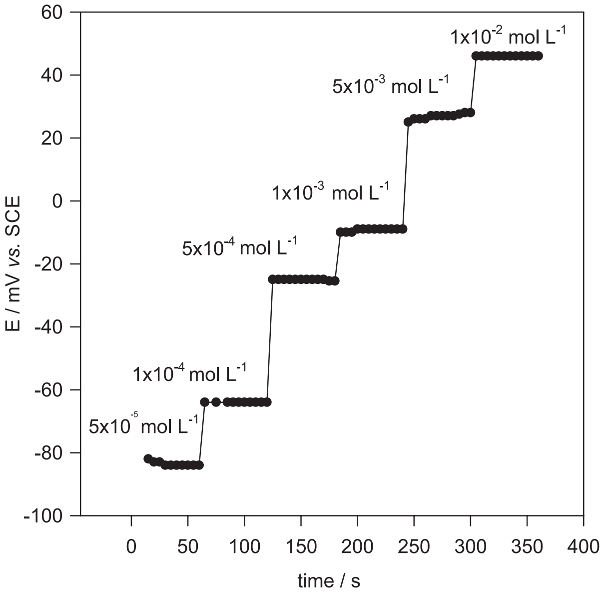


Figure 3. Typical potential-time plot for the response of oxeladin plastic membrane electrode.

Effect of temperature of the test solution

To study the thermal stability of the investigated electrodes, calibration graphs were constructed at different test solution temperatures, and the isothermal coefficients (dE/dt) of these electrodes were calculated,[23] to be −1.54 and −1.96 mV ^{o}C^{-1} for the plastic and carbon paste electrodes, respectively.

Optimization of the electrodes response in FI conditions

The FI techniques have found wide applications recently mainly due to reduction of the analysis time and reagents consumption compared to conventional manual procedures. The parameters were optimized in order to obtain the best sensitivity. The dispersion coefficients were found to be 1.38, 1.55 and 1.42 in the case of plastic membrane, carbon paste and MWCNT composite coated copper wire electrodes, respectively, these limited dispersion coefficients aid the optimum sensitivity and fast response of the electrodes.[24] The effect of flow rate on the electrode response was studied at different flow rates (4.15-30.0 mL min^{-1}) using 10^{-3} mol L^{-1} oxeladin citrate with constant injection sample loop size of 75 µL. The residence time of the sample is inversely proportional to the flow rate.[25] It was found that as the flow rate increases the peak becomes higher and narrower until flow rates of 12.50, 17.85 and 12.50 mL min^{-1} are reached in the case of plastic, carbon paste and MWCNT composite coated copper wire electrodes, respectively. The peaks obtained using flow rates greater than these flow rates were nearly the same. These flow rates were used in this work providing the maximum peak height, shorter time to reach the base line and less consumption of the carrier solution. The effect of injected sample volumes (4.7-500 µl) was studied by injecting 1×10^{-3} mol L^{-1} oxeladin citrate into the FI unit at the previously selected flow rates. Peak heights increased with the increase of the injected volumes. A 150 µL sample loop was used with each of the three sensors. This provides a good compromise between sensitivity and speed of analysis.

Under these conditions, the performance characteristics are slope 66.6, 70.3 and 61.6 concentration $decade^{-1}$, linear range of 2×10^{-5} - 1×10^{-2}, 1×10^{-5} - 1×10^{-2} and 5×10^{-5} - 1×10^{-2} mol L^{-1} for plastic membrane, carbon paste and MWCNT composite coated copper wire electrodes, respectively. Figure 4 represents a typical recording and the calibration graphs for plastic membrane as representative figure.

Validation of the proposed methods

Selectivity

The effect of some inorganic cations, sugars, amino acids, vitamins and urea on the response of oxeladin sensors were investigated according to IUPAC recommendations using the separate solution method (SSM)[16] or matched potential method (MPM).[17] The selectivity coefficient values ($-\log K^{pot}_{Oxeladin, J^{z+}}$) of the developed electrodes listed in Table 4 indicate that these electrodes are highly selective to oxeladin cation under both batch and FI conditions. The high selectivity in the case of inorganic cations is related to the difference in their mobility and permeability as compared to oxeladin cation, and in the case of sugars, amino acids and urea to the difference in polarity and lipophilic nature of their molecules relative to oxeladin cation. In FI measurements, the sample remains in contact with the electrode for a short time, consequently the selectivity coefficients must be different from that found in batch conditions.

Precision

The intra-day and inter-day (day-to-day) precisions expressed by relative standard deviations were 1.37 and 1.97% (n = 4), 1.14 and 1.71% (n = 4) and 0.79 and 1.06% (n = 4), for plastic membrane, carbon paste and MWCNT composite coated copper wire electrodes, respectively.

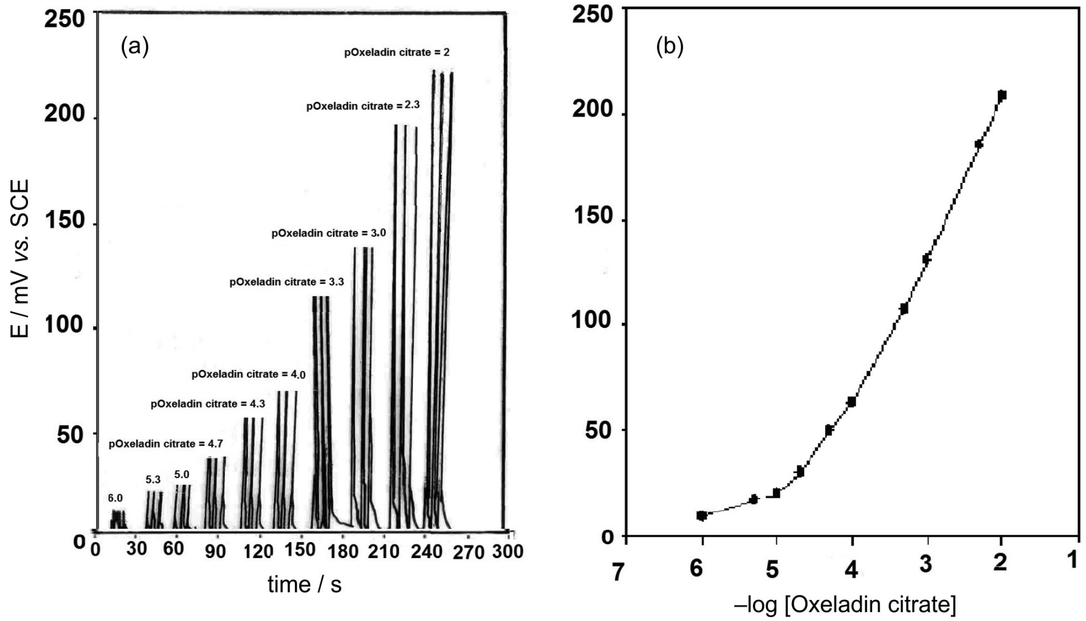


Figure 4. Recording (a) and their corresponding calibration graph (b) for oxeladin plastic membrane electrode under FI conditions

Table 4. Selectivity coefficients for the oxeladin electrodes in batch and flow injection (FI) conditions

Interferent	$-\log K^{pot}_{Oxeladin, J^{z+}}$					
	Plastic membrane electrode			Carbon paste electrode		
	Batch		FI	Batch		FI
	SSM	MPM		SSM	MPM	
Na^+	1.53	–	1.63	1.44	–	1.19
K^+	1.73	–	1.58	1.58	–	1.25
Ca^{2+}	3.46	–	2.72	3.10	–	2.49
Mg^{2+}	3.44	–	2.66	3.19	–	2.43
Zn^{2+}	3.48	–	2.78	2.50	–	1.93
Co^{2+}	3.37	–	2.75	2.80	–	1.97
NH_4^+	2.01	–	1.71	1.54	–	1.23
VitaminB$_1$	2.07	–	0.61	1.18	1.42	0.64
VitaminB$_6$	1.99	–	1.46	1.42	1.42	0.85
Glucose	–	3.12		–	2.93	
Fructose	–	3.14		–	2.78	
Lactose	–	3.13		–	2.89	
Maltose	–	2.99		–	2.86	
Glycine	–	3.06		–	2.66	
Alanine	–	3.03		–	2.73	
Urea	–	3.07		–	2.73	

SSM: separate solution method; MPM: matched potential method.

Robustness

The robustness[26] was examined by evaluating the effect of small changes in pH (4.7-5.3) of 0.1 mol L^{-1} acetate buffer on the recovery and standard deviation of the developed methods. The recovery and standard deviation values were not significantly affected by these variations, consequently the optimized procedures were reliable for assay of oxeladin citrate and could be robust.

Ruggedness

Ruggedness[26] was examined by using two different mV-meter, Seibold G-103 digital pH/mV-meter, Vienna, Austria and a digital Schott Geräte pH/mV-meter, model CG820, were used for applying the proposed procedures to assay oxeladin citrate under the same experimental conditions. The results obtained were reproducible since there is no significant difference between recovery and standard deviation values.

Analytical applications

The developed sensors were applied in the potentiometric determination of oxeladin citrate in bulk and the pharmaceutical preparation (Paxeladine® capsules)

Table 5. Determination of oxeladin citrate in pure form and pharmaceutical preparations by applying standard addition method and FI technique

	Plastic membrane electrode			Carbon paste electrode			MWCNT modified copper coated electrode		
	Taken / (mol L^{-1})	Recovery / %	RSD / %	Taken / (mol L^{-1})	Recovery / %	RSD / %	Taken / (mol L^{-1})	Recovery / %	RSD / %
Standard addition Pure solution	1×10^{-5}	99.86	0.458	5×10^{-5}	99.82	0.185	1×10^{-5}	97.76	0.610
	5×10^{-5}	99.41	0.519	1×10^{-4}	97.39	1.774	5×10^{-5}	97.56	1.189
	1×10^{-4}	99.33	1.494	2×10^{-4}	99.88	1.105	1×10^{-4}	99.97	1.883
	2×10^{-4}	99.41	1.026	5×10^{-4}	97.78	0.950	2×10^{-4}	97.97	1.124
Standard addition Paxeladine capsules	1×10^{-5}	99.92	0.368	5×10^{-5}	97.74	0.675	1×10^{-5}	97.64	0.663
	5×10^{-5}	97.59	0.446	1×10^{-4}	98.65	0.205	5×10^{-5}	98.73	1.233
	1×10^{-4}	97.48	0.633	2×10^{-4}	97.11	1.024	1×10^{-4}	99.53	0.702
	2×10^{-4}	98.36	0.827	5×10^{-4}	98.73	1.202	2×10^{-4}	99.31	1.302
FI	1×10^{-4}	100.00	1.404	1×10^{-4}	97.29	1.496	1×10^{-4}	98.99	1.460
	2×10^{-4}	97.14	1.549	2×10^{-4}	99.61	1.120	2×10^{-4}	98.18	1.188
	5×10^{-4}	98.31	1.427	5×10^{-4}	99.66	0.653	5×10^{-4}	98.05	0.617

RSD: relative standard deviation.

in batch and FI condition. (Table 5). The results were compared to those obtained from previously reported HPLC method.[8] The results are illustrated in Table 6. The data indicate a satisfactory agreement between the oxeladin citrate content determined by the developed sensors and the reference published HPLC method. Statistical comparison of the accuracy and precision of the developed methods with the reference published HPLC method (Table 6) was performed using student's *t*-test and the *F*-ratio tests at a 95% confidence level.[27] The *t*- and *F*-values did not exceed the theoretical values.

Table 6. Statistical comparison between the results of the pharmaceutical preparation paxeladine capsules on applying the proposed and reference methods

Parameter	Proposed method		Reference method [8]
	Standard addition	FI	
Plastic membrane electrode			
Mean recovery / %	98.17	98.48	97.54
SD / %	0.749	1.437	1.822
RSD / %	0.763	1.459	1.868
F-ratio (9.28)	5.917	1.608	
t-test (2.447)	0.640	0.810	
Carbon paste electrode			
Mean recovery / %	98.06	98.85	
SD / %	0.761	1.075	
RSD / %	0.776	1.088	
F-ratio (9.28)	5.732	2.873	
t-test (2.447)	0.527	1.238	
MWCNT composite coated copper electrode			
Mean recovery / %	98.80	98.41	
SD / %	0.968	1.072	
RSD / %	0.980	1.088	
F-ratio (9.28)	3.543	2.889	
t-test (2.447)	1.221	0.823	

SD: standard deviation; RSD: relative standard deviation.

The determination of oxeladin citrate in spiked human urine samples was carried out at two different levels of concentrations in batch conditions using the developed sensors. The mean recovery and relative standard deviations were calculated and summarized in Table 7.

Table 7. Determination of oxeladin citrate in spiked urine samples by applying standard addition method (n = 4)

	Taken / (mol L^{-1})	Recovery / %	RSD / %
Plastic membrane electrode	1×10^{-5}	99.43	0.563
	5×10^{-4}	97.95	1.625
Carbon paste electrode	6×10^{-5}	98.16	1.843
	5×10^{-4}	98.41	0.730
MWCNT composite coated copper wire electrode	1×10^{-5}	98.84	0.794
	5×10^{-4}	97.52	0.307

RSD: relative standard deviation.

Conclusion

Three types of potentiometric sensors were constructed. These were based on using oxeladin-phosphotungstate ion associate as the electroactive material. They were used for determination of oxeladin citrate in batch and flow injection conditions. The constructed sensors demonstrated advanced performance, with fast response, lower limit of detection and wide concentration range. The sensors were successfully applied in the determination of the drug in pharmaceutical formulation and spiked human urine samples. The multiwalled carbon nanotube composite coated copper wire electrode has lower limit of detection than plastic membrane and carbon paste electrodes, but the carbon paste electrode has a longer life time than plastic membrane and multiwalled carbon nanotube composite coated copper wire electrodes. The proposed methods are more sensitive than the published spectrophotometric method,[4] the official non-aqueous titration method[2], in the lower concentrations is difficult to determine in non-aqueous titration methods due to the small pH ranges which usually take place as titration ends in these methods.[28] The proposed methods are also less expensive than the published GC-MS[7] and HPLC[8] methods, which need expensive instruments and expensive HPLC grade solvents which are hazardous on environmen.

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Submitted: October 1, 2012