Development of New Sensors for Determination of Escitalopram Oxalate in Dosage Forms and Biological Fluids

Fatimah M.G.Al-Amri[*]*, Nawal A. Alarfaj and Fatma A.Aly*

Department of Chemistry, College of Science, King Saud University, P.O. Box 22452, Riyadh 11495, Saudi Arabia
[*]E-mail: chimstary@hotmail.com

A simple, accurate and precise potentiometric method was developed for the determination of escitalopram oxalate (ESC-OX) in pure form, its pharmaceutical formulations and biological fluids. Three types of sensors: plastic membrane, coated wire and coated graphite rod were constructed from the incorporation of escitalopram (ESC) with phosphomolybdic acid (PMA) or ammonium reineckate (ARK) or phosphotungstic acid (PTA) for the three sensors, respectively. Different parameters affecting the performance of the sensors were studied as pH, soaking time and the selectively of the sensors towards several foreign substances. The sensors exhibit linear response with a Nernstian slope (58.50 ±0.42, 57.80 ± 0.69 and 57.97 ± 0.52 mV decade $^{-1}$ at 25 ºC) over the concentration ranges 1.0×10^{-6}-1.0×10^{-2}, 1.0×10^{-6}-1.0×10^{-2} and 1.0×10^{-7}-1.0×10^{-2} mol L^{-1}, for the three sensors, respectively. The working pH range of the proposed sensors was 3-7. The influence of possible interfering species such as common inorganic cations, sugars and amino acids was studied. The proposed method was validated for linearity, accuracy, intra-day and inter-day precision, robustness and ruggedness according to ICH guidelines. Statistical student's t-test and F-test showed no significant difference between the proposed and reference methods regarding accuracy and precision, respectively.

1. INTRODUCTION

Escitalopram oxalate is chemically known as S-(+)-1-(3-dimethyl-aminopropyl)-1-(4-fluorophenyl)-1,3-dihyroisobenzofuran-5-carbonitrile oxalate,(Figure 1).It is an antidepressant of the selective serotonin reuptake inhibitor class, which help to normalize the levels of serotonin in the brain [1, 2]. The mechanism of antidepressant action of ESC is presumed to be linked to potentiation of serotonergic activity in the central nervous system resulting from its inhibition of CNS neuronal

reuptake of serotonin (5-hydroxytryptophan, 5-HT). In vitro and in vivo studies in animals suggest that ESC is a highly selective serotonin reuptake inhibitor with minimal effects on norepinephrine and dopamine neuronal reuptake [3].
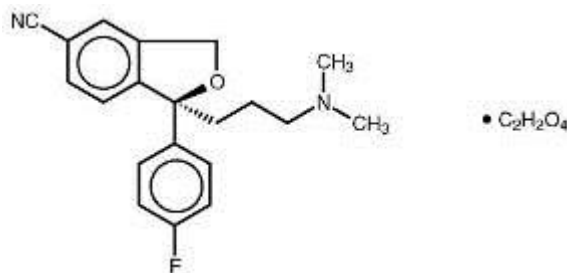
Figure 1. Chemical structure of escitalopram oxalate

Several methods have been reported for the determination of (ESC-OX) including high performance thin layer chromatography (HPTLC) [4,5], high performance liquid chromatography with ultraviolet detection (HPLC/UV) [6-10], liquid chromatography with mass spectrometry (LC/MS) [11,12], capillary electrophoresis [13,14], spectrophotometry[15-19] spectrofluorimetry [20, 21]   and chemiluminescsence [22].

The advantages of ion selective electrodes (ISEs) are simple to design, small size sensors, short measurement time, low cost, adequate precision, high accuracy, low detection limits and wide analytical ranges. The use of ISEs is increasing day by day in medicinal, pharmaceutical, environmental, agricultural and industrial fields [23-25]. In the present study three sensors have been constructed and validated for the determination of (ESC-OX) in pure drug, its pharmaceutical preparations and biological fluids.

2. EXPERIMENTAL

2.1. Instrumentation

The electrochemical measurements were carried out with HANNA instrument pH-211 microprocessor pH-meter and Metrohm pH-meter Model 744 for measuring pH . Ag/AgCl electrode was used as an external reference electrode.

The effect of pH on the potential of the electrode(s) was measured using two pH/mV meters. The combined glass calomel electrode was connected to one instrument and the ESC-electrode(s) with the double junction Ag/AgCl reference electrode was connected to the second instrument.

2.2. Materials and Reagent

All chemicals used were of analytical reagent grade and distilled water was used throughout. Pure grade ESC-OX was kindly supplied from Saudi Pharmaceutical Industries and Medical

Appliances Corporation (SPIMACO), Al-Qassim Pharmaceutical Plant, Saudi Arabia. The Pharmaceutical preparation Cipralex® tablets (10 mg ESC/tablet) were manufactured by H. Lundbeck A/S (Denmark) and were purchased from commercial sources. Tetrahydrofuran (THF) 97.0% and dioctyl phthalate (DOP) 99.0% were provided by Fluka – Switzerland. Poly vinyl chloride (PVC) high molecular weight, phosphomolybdic acid (PMA) 99.99% phosphotungstic acid (PTA) 99.99% and ammonium reineckate (ARK) 95.00% were supplied by Aldrich, Germany. Urine samples were obtained from healthy volunteers. Serum samples (Multi -Serum Normal, Ranbdox laboratories UK) were obtained from commercial sources.

2.3. Standard Drug Solutions

A stock solution containing 0.1 mol L^{-1} of ESC-OX was prepared daily by dissolving 1.035 g of drug in 25 mL distilled water. Working standard solutions ranging from 1.0×10^{-6}-1.0×10^{-2}, 1.0×10^{-6}-1.0×10^{-2} and 1.0×10^{-7}-1.0×10^{-2} mol L^{-1}, for the three sensors, ESC-PM, ESC-RK and ESC-PT, respectively were prepared from stock solution by appropriate dilution with distilled water.

2.4. Preparation of Escitalopram Ion-pair and Membrane Composition

The ion-pair was prepared by mixing 50 mL of ESC-OX 1.0×10^{-2} mol L^{-1} and 50 mL of 1.0×10^{-2} mol L^{-1} PMA or ARK or PTA. The resulting precipitates were filtered, washed thoroughly with distilled water and air dried. The membranes were prepared by dissolving 10 mg of the ion-pair, 190 mg of powdered PVC and 0.35 mL of DOP in 5 mL THF. The solution mixture was poured into a petri dish (3 cm diameter), covered with a filter paper and the solvent was allowed to evaporate slowly at room temperature.

## 2.5. Electrode Construction

2.5.1. Plastic membrane sensor:

A circular membrane was attached to a polyethylene tube (8.0 mm diameter) in electrode configuration by means of PVC-THF solution. A mixture containing equal volume of 1.0×10^{-3} ESC-OX and 1.0×10^{-3} mol L^{-1} potassium chloride was used as internal solution in which the Ag/AgCl reference electrode was dipped. The prepared electrode was pre-conditioned after preparation by soaking for at least 24 h in 1.0×10^{-3} mol L^{-1} ESC-OX and stored in the same solution. All potentiometric measurements were performed using the following cell assembly:

Ag/AgCl / Internal solution / membrane / test solution // KCl salt bridge // Ag/AgCl.
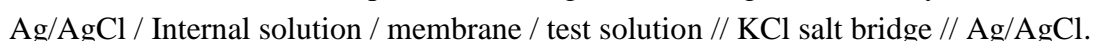
2.5.2. Coated wire sensor:

Coated wire sensor was fabricated using pure aluminum wire of 4.0 cm length and 0.5 cm diameter which tightly insulated by polyethylene tube leaving 1.0 cm at one end for the coating and 0.5 cm at the other end for the connection. Prior to coating , the polished aluminum surface was washed

with a detergent and water, and then dried with acetone. The wire was rinsed with chloroform and allowed to dry in air. The wire then coated by quickly dipping it into the coating solution (described previously under membrane composition) several times, and allowing the film left on the wire to dry for 5 min. The process was repeated several times until a plastic membrane was formed. The constructed electrode was preconditioned by soaking for 24 h in 1.0×10^{-3} mol L^{-1} of ESC-OX. All potentiometric measurements were performed using the following cell assembly:

Al / membrane / test solution // KCl salt bridge // Ag/AgCl.
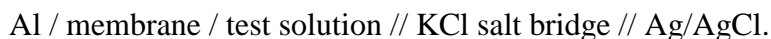
2.5.3. Coated graphite sensor:

A pure graphite rod of 4.0 cm length and 4.0 mm diameter was insulated by tight polyethylene tube, leaving 1.0 cm at one end for coating and 0.5 cm at the other end for connection. The polished sensor surface was coated with the active membrane by dipping the exposed end into the coating solution (described previously under membrane composition) ten times and allowing the film left on graphite rod to dry in air for 1 min each time. The process was repeated until 1.0 mm thickness formed [25]. The prepared electrode was preconditioned by soaking for 24 h in 1.0×10^{-3} mol L^{-1} ESC-OX . All potentiometric measurements were performed using the following cell assembly:

Graphite rod / membrane / test solution // KCl salt bridge // Ag/AgCl.

2.6. Electrodes Calibration

50.0 mL aliquots of ESC-OX standard solutions ranging from 1.0×10^{-2}-1.0×10^{-6}, 1.0×10^{-6}-1.0×10^{-2} and 1.0×10^{-7}-1.0×10^{-2} mol L^{-1}, were transferred into 100 mL beaker for the three sensors, ESC-PM, ESC-RK and ESC-PT, respectively. The sensor(s) and Ag/AgCl reference electrode were immersed in each solution and the potential was measured. The sequence of measurements was carried out from low concentration to higher one. The sensor(s) was washed with distilled water and dried with tissue paper between measurements. The measured potential was plotted against the negative logarithm of drug concentrations. Alternatively, the corresponding regression equation was calculated.

2.7. Standard Addition Method

The fabricated sensor was immersed into sample of 50 mL with unknown concentration (ca. 1.0×10^{-4} mol L^{-1}) and the equilibrium potential of E_1 was recorded. Then 0.1 mL of 0.1 mol L^{-1} of standard drug solution was added into the testing solution and the equilibrium potential of E_2 was recorded. The concentration of the testing sample was calculated from the change of potential ΔE (E_2-E_1).

2.8. Electrode Selectivity

Selectivity coefficients $K^{Pot}_{ESC, j^{+z}}$ of the sensors towards different cations, sugars and amino acids were determined by the separate solution method [26] in which the following equation was applied:

Log $K^{Pot}_{ESC} J^{z+} = (E_2 - E_1)/ S + \log [ESC] - \log [J^{z+}]^{1/z}$

Where, E_1 is the electrode potential in 1.0 x 10^{-3} mol L^{-1} ESC-OX solution, E_2 is the potential of the electrode in 1.0 x 10^{-3} mol L^{-1} solution of the interferent ion J^{z+} and S is the slope of the calibration plot in mV.

2.9. Effect of pH

The effect of pH of 1.0 x 10^{-4} mol L^{-1} ESC-OX solution on the sensor(s) potential was investigated. The variation of potential as a function of pH was followed by the addition of small volumes 0.1 mol L^{-1} of hydrochloric acid or sodium hydroxide to adjust the pH of this solution between 2-9. The potential readings corresponding to different pH values were recorded and plotted using the proposed sensor(s)

2.10. Determination of ESC-OX in Pharmaceutical Dosage Forms

2.10.1. Determination of ESC-OX in tablets

Table 1. Critical response characteristics of ESC sensors

Parameters	ESC-PM	ESC-RK	ESC-PT
Slope (mV decade^{-1})	58.50±0.42	57.80±0.69	57.97±0.52
Intercept	687.20	556.60	636.00
Correlation coefficient, r	0.9999	0.9999	0.9999
Linear range of drug conc., mol L^{-1}	1.0x10^{-6}-1.0x10^{-2}	1.0x10^{-6}-1.0x10^{-2}	1.0x10^{-7}-1.0x10^{-2}
LOD, mol L^{-1}	5.0x10^{-7}	4.8 x10^{-7}	5.0x10^{-8}
Working pH range	3-7	3-7	3-7
Response time for 1.0x10^{-4} M ESC/s	40	45	45
Lifetime/day	45	40	50
Robustness[a]	99.52±0.45	99.58±0.35	99.51±0.64
Ruggedness[b]	99.62±0.27	99.61±0.39	99.61±0.38

[a]Small variation in method parameters were carried out as pH of phosphate buffer (pH 6±1).
[b]Comparing the results by those obtained by different sensors assemblies using (Jenway 3510 pH meter)

25 tablets of Cipralex[®] (10 mg ESC/tablet) were finely powdered and an accurately weighed amount containing 0.207 g of (ESC-OX) was transformed into a 50-mL volumetric flask, and completed to the mark with distilled water. The flask with its contents was sonicated for 20 min and then filtered. Working solutions were prepared in the range of each sensor cited in Table 1 by appropriate dilution with distilled water. These solutions were analyzed as described above under sensor(s) calibration and standard addition methods.

2.10.2. Content uniformity assay of ESC-OX tablets:

Ten individual tablets of Cipralex[®] (10 mg ESC/tablet) were placed each separately in 100-mL measuring flask and dissolved in 50 mL distilled water then completed to volume with distilled water.

The sensor(s) was directly immersed into 10 mL of each sample for five times and then washed with distilled water to reach steady potential between the individual measurements. The mean potential was used to evaluate the content uniformity from the calibration graph or the regression equation.

2.11. Application to Serum and urine

2.11.1. Urine

1.0 mL aliquots of urine were transferred into a seires of 100-mL measuring flasks. Aliquots of ESC-OX standard aqueous solution were added so that the final concentration is in the range cited in Table 1. The flasks were mixed well and completed to volume using distilled water. These solutions were analyzed as described above under electrode calibration and standard addition methods.

2.11.2. Serum

1.0 mL aliquots of serum were transferred into a series of centrifugation tubes. Aliquots of ESC-OX standard aqueous solution were added so that the final concentration is in the range cited in Table 1. The tubes were mixed well and 10.0 mL of diethyl ether was added to each tube and centrifuged for 2 min at 1500 rpm. Then, the deproteinated layer was transferred to a 100-mL measuring flask and complete to volume using distilled water. These solution were analyzed as described above under electrode calibration and standard addition methods.

3. RESULTS AND DISCUSSION

3.1. Membrane Composition

The membrane was prepared using 2% ion pair, 35% PVC and 63% DOP for the three sensors. This membrane composition was reported previously for some sensors used in pharmaceutical analysis [27-30].

3.2 Nature and Response Characteristics of the Sensors

ESC-OX reacts with PMA, ARK and PTA to form a stable phosphomolybdate (ESC-PM) or reineckate (ESC-RK) or phosphotungstate (ESC-PT) ion-pair complex which is water insoluble but readily soluble in an organic solvents such as (THF). The ion-pair complex was prepared and tested as an active material with DOP as a solvent mediator in a PVC membrane matrix for ESC-OX. The choice of THF membrane solvent to achieve the required selectivity was based on its electric permittivity, immiscibility with aqueous phase, high viscosity, low solubility of the matrix in the membrane and its ability to dissolve the ion-pair complex.
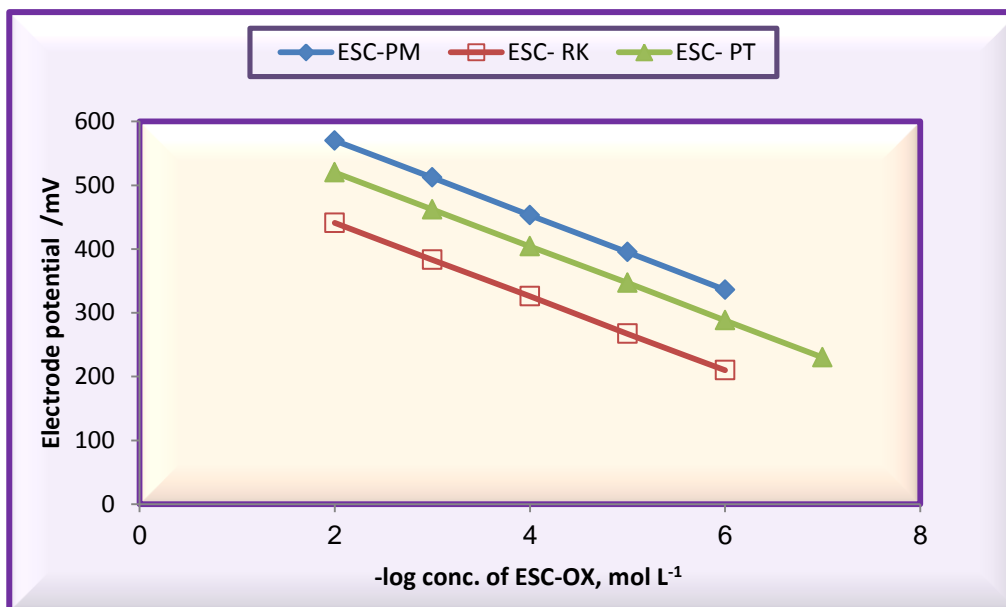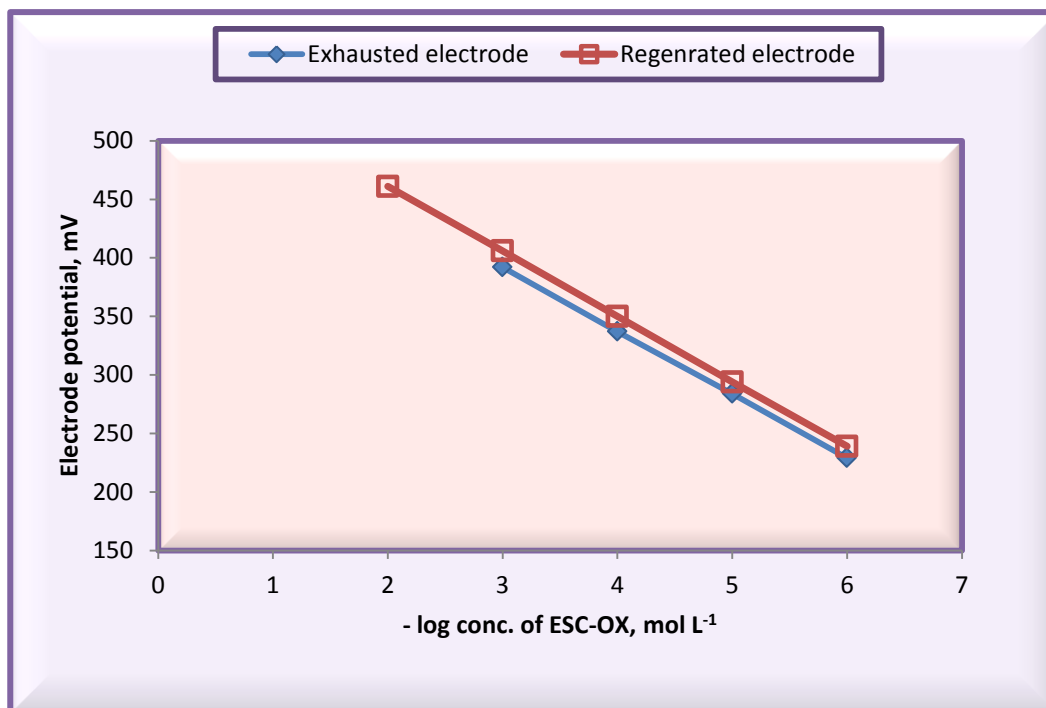
Figure 2. Calibration graphs of ESC sensors

The critical response characteristics of ESC-PM plastic membrane sensor, ESC-RK coated wire sensor and ESC-PT coated graphite sensor were determined and the results are summarized in Table 1. The sensors exhibits a near-Nernstian response over the concentration range from 1.0×10^{-6}- 1.0×10^{-2} , 1.0×10^{-6}- 1.0×10^{-2} and 1.0×10^{-7}- 1.0×10^{-2} mol L^{-1} ESC-OX for ESC-PM, ESC-RK and ESC-PT sensors , respectively, with a slope of 58.50 ± 0.42, 57.80 ± 0.69 and 57.97 ± 0.52 mV decade^{-1} at 25℃ for the previously mentioned sensors , respectively as in (Figure 2). The sequence of measurements was from low to high concentrations. The sensors exhibit a fast dynamic response of about 40, 45 and 45 s for ESC-PM, ESC-RK and ESC-PT sensors, respectively. The sensors were used for period of 45, 40 and 50 days for the previously mentioned sensors, respectively, without significant change in the sensors parameters. During this period, the sensors were stored in 1.0×10^{-3} mol L^{-1} drug solution.

3.3. Effect of Soaking Time and Regeneration of the Electrode

The performance characteristics of ESC sensor(s) was studied as a function of soaking time. For this purpose the sensor(s) was soaked in 1.0×10^{-3} mol L^{-1} ESC-OX solution and the calibration graphs were plotted after optimum soaking of time 24 h for ESC-PM, ESC-RK and ESC-PT sensors, respectively. The slope of the calibration curves was 58.50 ± 0.42, 57.80 ± 0.69 and 57.97 ± 0.52 mV decade-^{1}, at 25ºC for the three sensors, respectively. The sensor(s) was continuously soaked in 1×10^{-3} mol L^{-1} ESC-OX solution for 5, 15, 25, 35, 40, 45 and 50 days. The calibration plot slopes decreased

Figure 3. Calibration graphs of exhausted and regenerated ESC-PM membrane electrode

slightly to 56.90 ± 1.27, 56.30± 1.06 and 55.30 ±1.01mV decade^{-1} after 35 days for ESC-PM, ESC-RK and ESC-PT sensors, respectively, and continued to decrease reaching 54.00 ± 0.85 , 53.50±1.27 and 54.40±1.03 mV decade^{-1} after 45,40 and 50 days for the three sensors, respectively. This reveals that soaking of the sensor(s) in the drug solution for a long time has a negative effect on the response of the membrane.
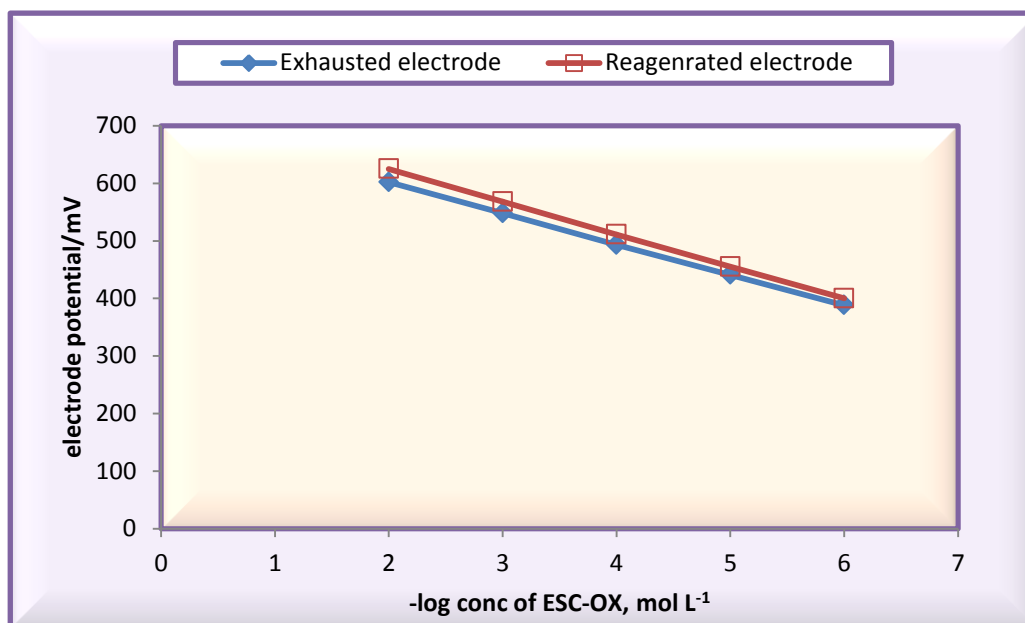
Figure 4. Calibration graphs of exhausted and regenerated ESC-RK coated wire electrode
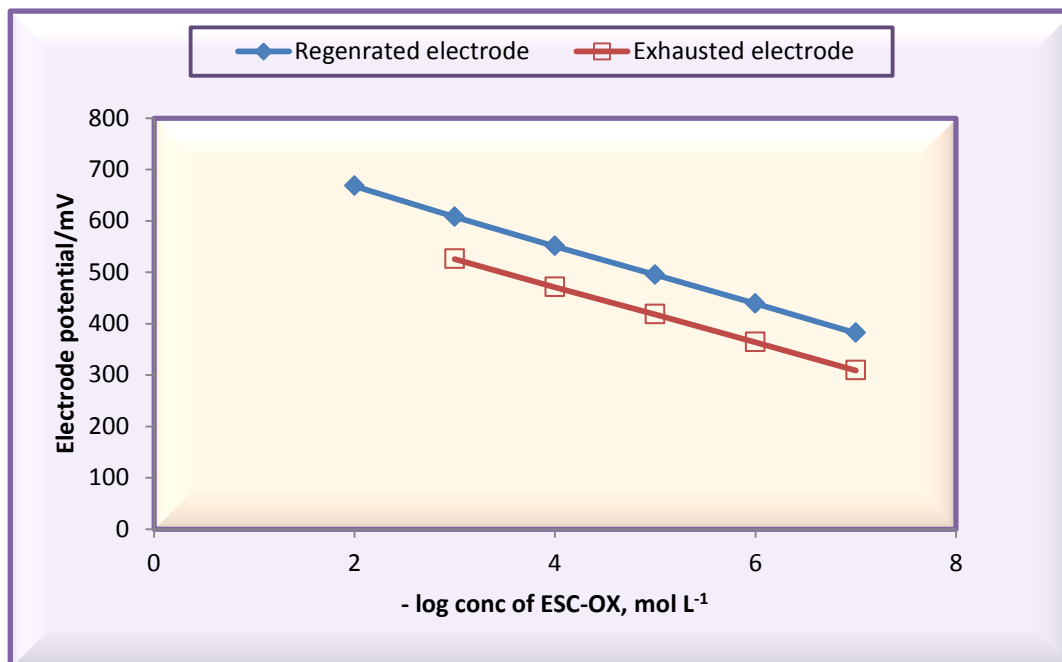
Figure 5. Calibration graphs of exhausted and regenerated ESC-PT coated graphite electrode

The same effect also appears after working with the sensors for a long time. The regeneration of the sensor(s) was tried simply by reformation of the ion-pair on the external gel layer of membrane [31]. The regeneration of the ESC membrane was successfully achieved by soaking the exhausted sensor(s) for 24 h in a solution that was 1.0×10^{-2} mol L^{-1} PMA or ARK or PTA, followed by soaking for 3 h in 1.0×10^{-2} mol L^{-1} ESC-OX solution. Figures 3-5 show the calibration graphs for an exhausted sensor(s) (slopes 54.00 ± 0.85, 53.50 ± 1.27 and 54.40 ± 1.03 mV decade^{-1}) for ESC-PM, ESC-RK and ESC-PT sensors respectively, and for the same sensor(s) after regeneration (slopes 55.60 ± 0.49, 54.30 ± 0.81 and 56.20 ± 0.56 mV decade^{-1}). It was found that the lifespan of the regenerated sensor(s) is limited to 8 h due to the ease of leaching of the lipophilic salts from the gel layer at the sensor(s) surface compared with those that are attached homogeneously to the PVC network through the solvent mediator.

3.4. Effect of pH

The effect of pH of the test ESC-OX solutions (1.0×10^{-4} mol L^{-1}) on the sensor(s) potential was investigated by measuring the variation in the electromotive force with change in pH gradually from 2-9. Figure 6 showed that within the pH range 3-7 the sensor(s) potential was practically independent of pH and in this range the sensor(s) can be safely used. Below pH 3, the electrode potential decreased with the increase of analyte acidity which may be attributed to extraction of H^+ ions by membrane. While at pH more than 7, the electrode potential decreased which may due to increase of OH$^-$ concentration [32].
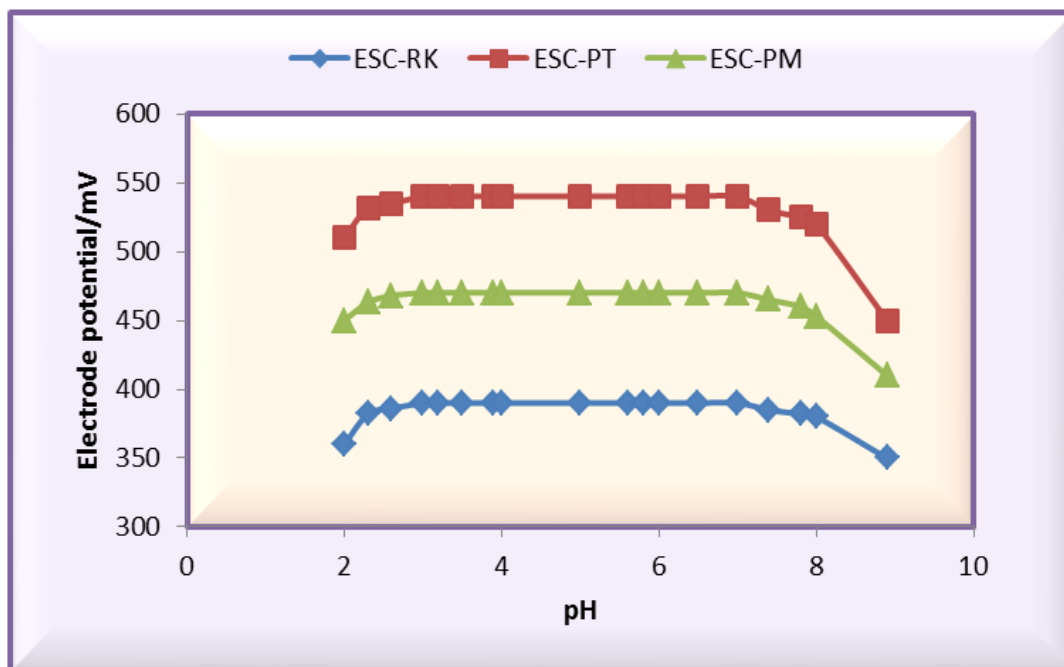
Figure 6. Effect of pH on ESC sensor potential

3.5. Selectivity of the Electrode

Table 2. Selectivity coefficients K^{pot} of ESC sensors towards different interferents

Interferent	$K^{pot}_{ESC,J^{z+}}$	$K^{pot}_{ESC,J^{z+}}$	$K^{pot}_{ESC,J^{z+}}$
	ESC-PM	ESC-RK	ESC-PT
Na^+	1.9×10^{-5}	2.1×10^{-4}	1.7×10^{-4}
K^+	1.3×10^{-5}	1.5×10^{-4}	2.8×10^{-5}
NH_4^+	3.5×10^{-4}	6.1×10^{-4}	2.9×10^{-3}
Ni^{2+}	8.4×10^{-6}	1.9×10^{-4}	2.0×10^{-5}
Zn^{2+}	2.6×10^{-5}	2.5×10^{-4}	1.3×10^{-4}
Mg^{2+}	3.2×10^{-5}	3.5×10^{-4}	4.6×10^{-4}
Ca^{2+}	3.4×10^{-4}	7.4×10^{-4}	1.7×10^{-3}
Sn^{2+}	1.6×10^{-4}	9.0×10^{-4}	2.4×10^{-3}
Ba^{2+}	1.9×10^{-4}	4.9×10^{-4}	1.9×10^{-3}
Cu^{2+}	3.7×10^{-5}	3.7×10^{-4}	1.1×10^{-3}
Mn^{2+}	4.2×10^{-5}	4.4×10^{-4}	1.3×10^{-3}
Cd^{2+}	1.6×10^{-5}	1.8×10^{-4}	6.6×10^{-5}
Thymidine	5.8×10^{-5}	2.9×10^{-3}	3.9×10^{-3}
L-histadin	7.3×10^{-5}	1.8×10^{-3}	6.2×10^{-3}
Ornithin	1.0×10^{-4}	1.1×10^{-3}	1.6×10^{-3}
L-glutamine	1.2×10^{-4}	6.5×10^{-4}	3.4×10^{-3}
Glycine	8.8×10^{-5}	1.8×10^{-3}	4.1×10^{-3}
L-Cystine	2.0×10^{-4}	3.2×10^{-3}	5.3×10^{-3}
Thymine	2.6×10^{-4}	2.2×10^{-3}	7.2×10^{-3}
Glucose	4.6×10^{-5}	5.2×10^{-3}	9.2×10^{-3}
Starch	5.9×10^{-4}	3.4×10^{-3}	7.3×10^{-3}
Talc	2.1×10^{-4}	7.4×10^{-3}	9.9×10^{-3}

The influence of some inorganic cations, sugars and amino acids on ESC sensor(s) was investigated. The results obtained (Table 2) reflect a very high selectivity of the investigated sensor for ESC. The mechanism of selectivity is mainly based on the stereospecificity and electrostatic environment, and is dependent on how much matching is present between the locations of the lipophilic sites in the two competing species in the bathing solution side and those present in the receptor of the ion-pair. The inorganic cations such as (Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+}, Sn^{2+} and Ba^{2+} did not interfere because of differences in the inorganic particle size, mobility and permeability. Also as shown in Table 2 the sensor(s) exhibits good tolerance towards sugars and amino acids.

3.6. Method Validation

The method was validated for linearity, accuracy, intra-day and inter-day precision, repeatability, robustness and ruggedness accordance with ICH guidelines [33].

Under the optimal experimental conditions, linear relationships exist between the sensor potential/mV and the negative logarithm of corresponding concentration of the investigated drug over the concentration range cited in Table 1. Also, the regression data and correlation coefficients (r) are listed in Table 1.

Table 3. Precision of the proposed method for the determination of ESC-OX in pure form

Taken –log conc. mol L^{-1}	ESC – PM			ESC - RK			ESC - PT		
	Recovery%	%RSD*	%SE**	Recovery%	%RSD*	%SE**	Recovery%	%RSD*	%SE**
Intraday Precision									
6.0	99.77±0.27	0.27	0.11	99.58±0.51	0.51	0.21	99.60±0.42	0.42	0.17
5.0	99.60±0.47	0.47	0.19	99.63±0.46	0.46	0.19	99.66±0.37	0.37	0.15
4.0	99.50±0.65	0.65	0.26	99.62±0.47	0.47	0.19	99.45±0.49	0.49	0.20
Interday Precision									
6.0	99.58±0.55	0.55	0.22	99.57±0.34	0.34	0.14	99.52±0.39	0.39	0.16
5.0	99.50±0.60	0.60	0.24	99.50±0.50	0.50	0.20	99.46±0.33	0.33	0.13
4.0	99.54±0.37	0.37	0.15	99.42±0.44	0.44	0.18	99.33±0.30	0.30	0.12

*%RSD= (S.D/Mean) 100
**%SE(%Error)= %RSD/√n

The detection limit of the investigated drug was calculated according to IUPAC [34] recommendation which stated that the detection limit is the concentration at which the measured potential differs from that predicted by the linear regression by more than 18 mV. The value reported

in Table 1; indicates that the proposed potentiometric method is sensitive for detection of very small concentrations of ESC-OX.

The robustness of the proposed method was carried out by using phosphate buffer pH 6±1 and the percentage recoveries were 99.52± 0.45, 99.58±0.35 and 99.51±0.64 for ESC-PM, ESC-RK and ESC-PT sensor(s), respectively. The ruggedness of the proposed method was investigated upon using another model of pH-meter (Jenway 3510) and the obtained results are shown in (Table 1).

The accuracy of the proposed method was evaluated by the recoveries studies for taken concentration of ESC-OX reference standards. The % recoveries values ranged from 99.28-99.98, 99.07-100.33 and 99.16-100.00 for ESC-PM, ESC-RK and ESC-PT sensors respectively, indicating the accuracy of the proposed method.

The precision of the method was calculated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of ESC-OX were analyzed in six replicates during the same day (intra-day precision) and for five consecutive days (inter-day precision). The % RSD values of intra-day and inter-day studies for the repeated determinations were less than 2% indicating good precision (Table 3).

3.7. Analytical Applications

3.7.1. The sensor response in pharmaceutical formulations

Table 4. Analysis of ESC in pure form and dosage forms by the proposed and reported methods

Sample	Statistical Parameter	ESC-PM		ESC-RK		ESC-PT		Reported method[18]
		Calibration Method	standard addition method	Calibration method	standard addition method	Calibration method	Standard addition method	
pure form	Mean ±S.D	99.59±0.27	99.60±0.57	99.75±0.43	99.76±0.33	99.62±0.44	99.73±0.24	99.69±0.42
	n	6	6	6	6	6	6	6
	Variance	0.08	0.32	0.19	0.11	0.19	0.06	0.18
	%SE**	0.12	0.23	0.18	0.14	0.18	0.10	0.17
	%RSD	0.28	0.57	0.43	0.33	0.44	0.24	0.42
	t-test	0.48(2.228)*	0.31(2.228)*	0.13(2.228)*	0.32(2.228)*	0.28(2.228)*	0.20(2.228)*	
	F-test	2.35(5.05)*	1.82(5.05)*	1.05(5.05)*	1.60(5.05)*	1.09(5.05)*	2.98(5.05)*	
Cipralex ® Tablets	Mean ±S.D	99.41±0.41	99.65±0.49	99.54±0.31	99.53±0.49	99.77±0.19	99.55±0.37	99.57±0.42
	n	6	6	6	6	6	6	6
	Variance	0.17	0.24	0.10	0.25	0.04	0.13	0.17
	%SE**	0.16	0.20	0.13	0.20	0.08	0.15	0.17
	%RSD	0.42	0.50	0.31	0.50	0.20	0.37	0.42
	t-test	0.67(2.228)*	0.30(2.228)*	0.14(2.228)*	0.15(2.228)*	0.77(2.228)*	0.05(2.228)*	
	F-test	1.02(5.05)*	1.39(5.05)*	1.78(5.05)*	1.43(5.05)*	2.17(5.05)*	1.17(5.05)*	

* The Figures in parentheses are the tabulated t- and F- tests at p = 0.05[35]
**%Error = %RSD/√n

It is evident from the above mentioned results that the proposed ESC-PM, ESC-RK and ESC-PT sensors gave satisfactory results with ESC-OX in its pure form (Table 4). Thus its pharmaceutical dosage form (Cipralex 10 mg /tablet) was subjected to the analysis for its ESC-OX content by the proposed potentiometric method. Table 4 shows the results of analysis of ESC-OX in its tablets. Statistical analysis [35] of the results obtained by the proposed and the comparison methods[18] shows no significant difference between the two methods as regards to accuracy (t-test) and precision (F-test). Also, the results in Table 3 indicate that the method is selective for the determination of ESC-OX in its pharmaceutical preparations without interference from excipients.

3.7.2. The sensor response in biological fluids

Sogaard et al. [36] reported that ESC-OX is relatively fast absorbed after oral administration of single or multiple doses. The maximum observed plasma or serum concentration (C_{max}) attained after 3 to 4 hours and was found to be about 65.0±14.5 nmol/L after   a single oral administration of 20 mg ESC to fasted subjects. The mean half-lives were 27 and 33 hours after single and multiple doses respectively.

The validity of the proposed method was further tested by analyzing ESC-OX in spiked biological fluids using the calibration and standard addition methods.

Table 5. Determination of ESC-OX in spiked human serum and urine by the ESC sensors

Sample	ESC-PM		ESC-RK		ESC-PT	
	Calibration Method*	standard addition method*	Calibration Method*	standard addition method*	Calibration Method*	standard addition method*
Urine	99.18±0.43	99.65±0.44	99.32±0.31	99.58±0.45	99.49±0.22	99.66±0.31
Serum	99.04±0.51	99.57±0.45	99.13±0.38	99.64±0.37	99.20±0.26	99.62±0.37

* Mean ± S.D. of six determinations

The results were listed in Table 5**.** The potential of the ESC sensors showed no significant difference of response time between aqueous solution of pure drug and its spiked biological fluids.

A comparison of the proposed method with other reported methods shows them all to be equally accurate and precise. However, HPLC methods are generally tedious procedures and require special skills and sophisticated instrumentation. Although the proposed method might be less sensitive than some methods [7, 20-22], it has a few merits such as simplicity, easy to operate, less expensive

and time efficiency. Moreover, the proposed method is more sensitive than some HPLC and spectrophotometric methods [8-10, 15-19].

4. CONCLUSION

The potentiometric method developed for the determination of ESC-OX using new three constructed sensors is simple, accurate, easy to operate and inexpensive; making it an excellent tool for the routine determination of ESC-OX in quality control laboratories. Also, it provides a fast assay of ESC-OX in its pharmaceutical preparations without interference from excipients.

References

1. http://en.wikipedia.org/wiki/Escitalopram
2. http://www.searlepak.com/morcet.html
3. http://www.rxlist.com/lexapro-drug.htm
4. M.V.Mahadeo, S.R. Dhaneshwar and M.J.Kulkarni, Euras. *J. Anal. Chem.* 2 (2007)101.
5. N. Dhavale, S. Gandhi, S. Sabnis and K. Bothara, *Chromatographia* 67 (2008) 487.
6. S.R. Dhaneshwar, M.V. Mahadeo and M. J. Kulkarni, *J. AOAC Int.* 92 (2008)138.
7. C. Greiner, C. Hiemke, W. Bader and E. Haen, *J. Chromatogr. B.* 848 (2007) 391.
8. S.V. Gandhi, N.D. Dhavale, V.Y. Jadhav and S.S. Sabnis*, J. AOAC, Int.* 91 (2008)33.
9. T. Samanta, S. Dey, H.B. Samal, D.B. Kumar, D.L. Mohanty and K. Bhar, Int. *J. Chem. Res.* 2 (2011)11.
10. S.B. Syama and A. Suneetha, *Int. J. Pharm. Bio. Sci.* 2 (2011) 140.
11. S.S. Singh, H. Shah, S. Gupta, M. Jain, K. Sharma, P. Thakkar and R. Shah, *J. Chromatogr. B.* 811 (2004) 209.
12. U. Gutteck and K.M. Rentsch, *Clin. Chem. Lab.Med.* 41 (2003) 1571.
13. N. Johannesson and J. Bergquist, *J. Pharm. Biomed. Anal.* 43 (2007) 1045.
14. B. Sungthong, P. Jac, and G.K. Scriba, *J. Pharm. Biomed. Anal.* 46 (2008) 959.
15. S. Sharma, H. Rajpurohit, C. Sonwal, A. Bhandari, V.R. Choudhary and T. Jain, *J. Yon. Pharm.* 2 (2010) 420.
16. T. Vetrichelvan, K. Arul, M. Sumithra and B. Umadevi, *Indian J. Pharm. Sci.* 72 (2010) 269.
17. S.A. Al-Tamimi, F.A. Aly and O.M. Al-Otaibi, *Asian J. chem.* 24 (2012)5515.
18. G. Bharat, B.G. Chaudhari and H.R. Parmar, *Int. J. Pharm. Qual. Assu.* 2 (2010)9.
19. S.A. Al-Tamimi, F.A. Aly, O.M. Al-Otaibi, *J. Indian Chem. Soc.* 90 (2013) (in press).
20. S.Victor, M. Alex, D. Vadim, A. Dan, V.Z. Louis and D. Anatoly, *Clin. Chem. Lab. Med.* 45 (2007) 513.
21. E.A. Taha, N.N. Salama and S. Wang, *Anal. Chem. Insights,* 4 (2009)1.
22. N. A. Alarfaj, F.A. Aly and A.A. Al-Qahtany, *Luminescence* 28 (2013)84.
23. A. M. Al-Mohaimeed, S. A. Al-Tamimi, N. A. Alarfaj and F.A. Aly, *Int. J. electrochem. Sci.* 7 (2012).
24. K. Zarzecka, M. Gugata and I. Mystkowska, *J. Toxoc. Environm. Health.* 73 (2010)1244.

25. S.I.M. Zayed, *Japan Soci. Anal. Chem.* 20 (2004)1043.
26. M. Arvand, M.F. Mousavi, M.A. Zanjanchi and M. Shamsipur, *J. Pharm. Biomed Anal.* 33 (2003)975.
27. M. S. Elazazy, A. Shalaby, M. N. Elbolkiny and H.M. Khalil, *Chin. Pharm. J.* 55 (2003) 491.
28. E. T. Maha, E.M. Magda and A..Shalaby, *Bull. Pharm. Sci.* 29 (2006) 488.
29. H. M. Elqudaby, E.Y.Z. Frag, G.G. Mohamed and M.A.E. Mohamed, *Anal. Bioanal. Electrochem.* 3 (2011) 420.
30. A.M. Badawey, A.E.B. Abd El-Aleem and N.T. Lamie, *Intentional J. of Comprehensive Pharmacy* 2 (2011) 1.
31. U. Oesh and W. Simon, *Anal. Chem.* 52 (1980) 692.
32. E. T. Maha, R. Sawsan, E.M. Magda and A.A. Shalaby, *Kor. J. chem.* 54 (2010) 1.
33. International Conference on Harmonization, ICH Harmonised Tripartite Guideline, Q2 (R1), Validation of Analytical Procedures: Text and Methodology, Switzerland, (1995).
34. Information Bull. IUPAC, No. 43, Recommendations for nomencleature of ion-selective electrodes, (1975).
35. J.C. Miller and J.N. Miller, Statistics for analytical chemistry, third ed., Ellis Horwood-Prentice Hall, Chichester, (1993).
36. B. Sogaard, H. Mengel, N. Rao, F. Larsen, *J. Clin. Pharmacol.* 45 (2005)1400.