SENSOR LETTERS
Vol. 9, 1–8, 2011

Potentiometric Determination of Cholesterol-Reducing Drug, Ezetimibe Using Coated Wire Membrane Sensors

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(Received: 1 March 2011. Accepted: 26 April 2011)

Newly developed coated wire membrane electrodes have been developed for determination of ezetimibe (EZ) in pure form, pharmaceutical preparations and in biological fluids. The selective electrodes were based on the incorporation of ezetimibe with the ion exchanger(s) phosphotungstic acid (EZ-PTS), phosphomolybdic acid (EZ-PMO) and a mixture of both (EZ-PTS/PMO). The electrodes exhibit linear response with a Nernstian slope (57.37 ± 0.235, 55.40 ± 0.748 and 58.04 ± 0.536 mV decade^{-1} at 25 °C) over concentration range $1.0 \times 10^{-6} - 1.0 \times 10^{-1}$, $1.0 \times 10^{-6} - 1.0 \times 10^{-2}$ and $1.0 \times 10^{-6} - 1.0 \times 10^{-1}$ mol L^{-1} for the three electrodes, respectively. The working pH range of the proposed electrodes was 3–7. The influence of possible interfering species such as common inorganic cations, many sugars, amino acids and different pharmacological related compounds was studied. Statistical student's *t*-test and *F* test showed insignificant systematic error between proposed and reported methods.

Keywords: Potentiometry, Ezetimibe, Coated Wire Membrane Electrode, Ion-Selective Electrodes, Pharmaceutical Preparations, Biological Fluids.

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1. INTRODUCTION

Ezeitimibe,[1] (3*R*, 4S)-1-(4-fluorophenyl)-3-[(3*S*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4 hydroxyphenyl)azetidin-2-one. (Fig. 1). It is used to lower cholesterol in people with high cholesterol levels (primary hypercholesterolaemia). It is normally used with another type of cholesterol-lowering medicine called a statin (for example simvastatin, atorvastatin), together with a low cholesterol diet and increased exercise. Ezetimibe is added to the statin treatment because it works in a different way to statins (which lower cholesterol by preventing its production by the liver) and so it can boost their cholesterol lowering effect.[2]

Several methods have been reported for the determination of ezetimibe viz., high performance liquid chromatography,[3–7] gas-chromatography-mass spectrometry,[8] liquid chromatography-mass spectrometry,[9–11] spectrophotometry,[12] and Potentiometry using glassy carbon nanotube electrode in which ezetimibe yields a well-defined anodic peak at the surface of the electrode in an aqueous solution of pH 13. A linear amperometric calibration curve was obtained in the range of 1.2–78 μM (0.5–32.0 μg/mL) of ezetimibe, with a sensitivity of 88.6 nA/μM and a detection limit of 300 nM (0.12 μg/mL).[13] Reviewing the literature revealed that, up to the present time, nothing has been published concerning the determination of ezetimibe in plasma by electrochemical sensors. The aim of this work is to develop a simple validated, sensitive, and time saving coated wire membrane electrodes for the determination of ezetimibe in pure form, pharmaceutical preparations and biological fluids.

2. EXPERIMENTAL DETAILS

2.1. Reagents and Materials

All chemicals used were of analytical grade, pure grade ezetimibe was kindly supplied from Saudi Pharmaceutical Industries and Medical Appliances Corporation, Al-Qassim Pharmaceutical Plant (SPIMACO), Saudi Arabia. The pharmaceutical preparation (Ezetrol® 10 mg/tablet) was provided by Schering-Plough Company, USA. Methanol 99.0%, Acetone 99.9%, Diethyl ether 99.0%, di-butyl phthalate (DBP) 99.0%, tetrahydrofuran (THF) 97.0% and toluene 99.5% were provided by Fluka, Switzerland. Polyvinyl chloride (PVC), high molecular

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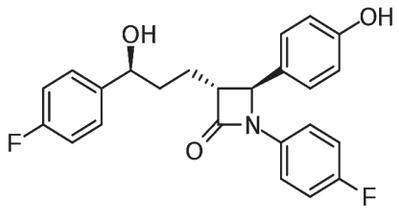

Fig. 1. Chemical Structure of Ezetimibe.

weight was purchased from Aldrich, Germany. Phosphomolybdic acid 98.80% and phosphotungstic acid 99.09% were provided by SOMATCO, Saudi Arabia. Urine samples were obtained from healthy volunteers and serum samples were obtained from commercial sources (Multi-Serum Normal, Randox Laboratories, UK).

2.2. Preparation of Ion-Exchangers

The ion-exchangers EZ-PTS and EZ-PMO were prepared by mixing stoichiometric amounts of 1.0×10^{-2} mol L^{-1} of ezetimibe in methanol with an equimolar solution of phosphotungstic or phosphomolybdic acid in distilled water, respectively. The resulting precipitates were filtered, washed thoroughly with distilled water and air dried. The chemical composition of the precipitates was identified and confirmed by (C, H, N) elemental analysis (Table I).

2.3. Membrane Composition

The membrane composition was studied by varying the percentages (w/w) of the ion exchange(s), polyvinyl chloride (PVC) and plasticizer di-butylphthalate (DBP), until an optimum composition that exhibiting the best performance characteristics was reached. The membranes were prepared by dissolving the required amount of PVC ion-exchanger(s) and the plasticizer (DBP) in 5.0 cm diameter Petri dish containing 10.0 mL tetrahydrofuran (THF). The Petri dish was covered with a filter paper and the solvent was allowed to evaporate slowly at room temperature.

2.4. The Electrodes

The electrodes were constructed as pure aluminum wire of 4.0 cm length was tightly insulated by polyethylene tube leaving 1.0 cm at one end for the coating and 0.5 cm at the other end for connection. Prior to coating, the polished aluminum surface was washed with a detergent, thoroughly rinsed with water, and dried with acetone. Then the wire was rinsed with chloroform and allowed to dry. Afterwards, the aluminum wire was 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 about 3 min. The process was repeated several times until a plastic membrane of approximately 1.0 mm thickness was formed. The prepared electrode was conditioned by soaking for 5 h in 1.0×10^{-3} mol L^{-1} ezetimibe solution.

Table I. Elemental analysis of the ezetimibe ion-associates.

	C%		H%		N%	
Ion-associate	Found	Calculated	Found	Calculated	Found	Calculated
I[a]	21.02	21.05	1.52	1.55	0.95	1.02
II[b]	30.35	30.24	2.18	2.22	1.44	1.47

Note: I[a] $[C_{24}H_{21}F_2NO_3]_3[P(W_3O_{10})_4]$; II[b] $[C_{24}H_{21}F_2NO_3]_3[PO_4 \cdot 12MoO_2]$.

2.5. The Electrochemical System

The electrochemical measurements were carried out with HANNA instruments pH 211 microprocessor pH-meter and Metrohm pH-meter Model 744 for measuring pH. Saturated calomel electrode (SCE) was used as an external reference electrode. All potentiometric measurements were performed using the following cell assembly: Al/membrane/test solution//KCl salt bridge//SCE.

2.6. Selectivity

The selectivity coefficients were determined by separate solution method[14] in which the following equation was applied:

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

Where $K^{Pot}_{Ez.}J^{z+}$ is the selectivity coefficient, E_1 is the electrode potential in 1×10^{-3} mol L^{-1} ezetimibe solution, E_2 is the potential of the electrode in 1×10^{-3} mol L^{-1} solution of interferent J^{z+} and S is the slope of calibration graph in mV.

2.7. Potentiometric Determination of Ezetimibe

2.7.1. Pure Drug

Ezetimibe has been determined potentiometrically using the investigated electrodes using $1.0 \times 10^{-6} - 5.0 \times 10^{-3}$, $1.0 \times 10^{-6} - 5.0 \times 10^{-4}$, and $1.0 \times 10^{-6} - 1.0 \times 10^{-1}$, for EZ-PTS, EZ-PMO and EZ-PTS/PMO, respectively.

2.7.2. Ezetrol® Tablets

For sampling of tablets, 20 tablets of Ezetrol® (10 mg/tablet) were finely powdered and appreciate weights were taken and dissolved in 25 mL methanol to prepare sample solutions ranging from $5.0 \times 10^{-6} - 1.0 \times 10^{-3}$ mol L^{-1} for direct determination and from $9.0 \times 10^{-6} - 5.0 \times 10^{-4}$ mol L^{-1} for standard addition method.[15]

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2.7.3. Content Uniformity Assay of Ezetrol® Tablets

Ten individual tablets of Ezetrol® (10 mg/tablet) were placed in separate 100 mL measuring flasks, dissolved in 25 mL methanol then completed to volume with distilled water. The electrode(s) was directly immersed into 10 mL of each sample for five times and should be washed with deionized water to reach steady potential between the individual measurements. The mean potential was used to evaluate the content uniformity from the calibration graph.

2.8. Application to Biological Fluids

Ezetimibe has been determined in human urine and serum using the investigated electrodes in the concentration range $3.0 \times 10^{-5} - 1.0 \times 10^{-3}$ and $5.0 \times 10^{-5} - 1.0 \times 10^{-3}$ mol L^{-1} for urine and serum, respectively.

3. RESULTS AND DISCUSSION

3.1. Composition of the Membranes

The amount of lipophilic salt should be sufficient to obtain reasonable ionic exchange at the gel layer/test solution interface, which is responsible for membrane potential.[16, 17] Also, the amount of plasticizer should be suitable for good physical properties and at the same time efficiently acts as a solvent mediator for the ion-exchange(s) lipophilic salts. An increase in the amount of plasticizer improves to a large extent the adhesive properties of the membrane but, it aids in the deterioration of the membrane. This depending on the properties of both the ion-exchanger(s) and the matrix.[18] In this work the ratio of plasticizer (DBP) to polymer was kept constant at 1:1, while the amount of ion-exchanger was varied.

Several compositions for the electrodes were investigated in which the ion-exchanger percentage ranged from 5–15% for EZ-PTS and EZ-PMO and from 5–20% for EZ-PTS/PMO of each ion-exchanger. The preparation process was highly reproducible as revealed by the low relative standard deviations values of the slopes obtained employing the prepared membranes (mean RSD was about 0.235%, 0.748% and 0.536%) for the above three mentioned electrodes, respectively.

The best performances were obtained using compositions of 10% EZ-PTS and EZ-PMO, 45% DBP and 45% PVC, while using 10% EZ-PTS, 10% EZ-PMO, 40% PVC and 40% DBP for EZ-PTS/PMO. The above optimum compositions were used to prepare membrane electrodes for all further investigations.

3.2. Nature and Response Characteristics of the Electrodes

Ezetimibe reacts with phosphotungstic acid and phosphomolybdic acid to form a stable phosphotungstate or phosphomolybdate ion-pair complex which is water insoluble but readily soluble in an organic solvent such as tetrahydrofuran. The complex was prepared and tested as active material with (DBP) as a solvent mediator in a polyvinyl chloride membrane response for ezetimibe. The critical response characteristics of coated wire electrodes were determined and results are summarized in Table II. The electrode(s) exhibited a Nernstian response over the concentration range from $1.0 \times 10^{-6} - 1.0 \times 10^{-1}$, $1.0 \times 10^{-6} - 1.0 \times 10^{-2}$ and $1.0 \times 10^{-6} - 1.0 \times 10^{-1}$ mol L^{-1} ezetimibe for EZ-PTS, EZ-PMO and EZ-PTS/PMO electrodes, respectively, with a slope of 57.37 ± 0.235, 55.40 ± 0.748 and 58.04 ± 0.536 mV $decade^{-1}$ at 25 °C for the previously mentioned electrodes, respectively as in Figure 2. The choice of THF membrane solvent to achieve the required selectivity is based on its electric permittivity and its immiscibility with aqueous phase, high viscosity, low solubility of the matrix in the membrane and ability to dissolve ion-pair complex.

3.3. Effect of Soaking

Freshly prepared electrodes must be preconditioned by soaking in 1×10^{-3} mol L^{-1} of drug test solution to form an active thin gel layer at which ion exchanger occurs. This preconditioning process requires different times depending on diffusion and equilibrium at the electrode test solution interface; a fast establishment of equilibrium is certainly a condition for a fast potential response.[19] For the present electrodes, the presoak times were 5, 6 and 6 h with slopes of 57.37 ± 0.235, 55.40 ± 0.748 and 58.04 ± 0.536 mV $decade^{-1}$ at 25 °C and a usable concentration range of $1.0 \times 10^{-6} - 1.0 \times 10^{-1}$, $1.0 \times 10^{-6} - 1.0 \times 10^{-2}$ and $1.0 \times 10^{-6} - 1.0 \times 10^{-1}$ mol L^{-1} for EZ-PTS, EZ-PMO and EZ-PTS/PMO, respectively.

Nevertheless, continuous soaking of the electrodes in 1×10^{-3} mol L^{-1} solution affects negatively their response to the ezetimibe; this was attributed to leaching of the active ingredients (ion-exchanger(s) and plasticizer) to the bathing solution. It was noticed that the slopes of the calibration graphs obtained by the preconditioned electrodes were nearly constant for the first 15 days; then they started to decrease gradually to about 55.10 mV $decade^{-1}$, after 20, 21 and 23 days, reaching about 51.50 mV $decade^{-1}$ after 25 days for EZ-PTS and EZ-PMO and 28 days for EZ-PTS/PMO. Figure 3 shows the effect of soaking on the EZ-PTS/PMO electrode.

The lifespan of the membrane containing the mixed ion-exchangers was much longer than that of the membrane containing an individual ion-exchanger. This can be correlated with some sort of physical interaction between the two ion-exchangers within the PVC network. It may also be due to the diffusion and partition coefficients of both the ion-exchangers and the plasticizer; there was a relatively large amount of ion-exchangers ($\approx 20\%$) in the

Table II. Critical response characteristics of ezetimibe sensors.

Parameter[a]	EZ-PTS	EZ-PMO	EZ-PTS/PMO
Slope (mV per decade)	57.37 ± 0.24	55.40 ± 0.75	58.04 ± 0.54
Intercept	738.13	1337.61	1268.55
Correlation coefficient r.	0.9999	0.9999	0.9999
Linear range (M)	$1.0 \times 10^{-6} - 1.0 \times 10^{-1}$	$1.0 \times 10^{-6} - 1.0 \times 10^{-2}$	$1.0 \times 10^{-6} - 1.0 \times 10^{-1}$
Detection limit (M)	2.5×10^{-7}	5.0×10^{-7}	1.9×10^{-7}
Response time for 10^{-3} M (s)	30.00	35.00	25.00
Working pH range	3.00–7.00	3.00–7.00	3.00–7.00
Lifetime/day	20.00	20.00	28.00
Accuracy (%)	99.14	98.93	99.22
Standard deviation	0.37	0.42	0.31
Repeatability %RSD	0.60	0.50	0.70
Between day variability %RSD	0.40	0.30	0.50
Robustness[b]	99.36 ± 0.34	98.92 ± 0.25	99.45 ± 0.48
Ruggedness[c]	99.41 ± 0.61	98.93 ± 0.61	99.43 ± 0.37

Notes. [a]Mean of three measurements; [b]A small variation in method parameters were studied as pH of buffer (phosphate buffer 6); [c]Comparing the results by those obtained by different sensors assemblies using Mettler Toledo pH-meter.

mixed membrane electrode compared to that present in the individual exchangers membrane electrodes.[20]

3.4. Regeneration of the Electrodes

The above discussion revealed that soaking the electrodes in the drug solution 1.0×10^{-3} mol L^{-1} for a long time has a negative effect on the response of the membranes. The same effect appears after working with the electrodes for a long time.

The regeneration of the electrodes was carried out simply by reformation of the ion-exchanger(s) on the external gel layer of the membrane. The regeneration of the EZ-PTS/PMO electrode was successfully achieved by soaking the exhausted electrode for 24 h in a solution that was 1.0×10^{-2} mol L^{-1} in both phosphotungstic acid and phosphomolybdic acid, followed by soaking for 3 h in 1.0×10^{-2} mol L^{-1} ezetimibe solution. Figure 4, shows the calibration graphs for the exhausted electrode (slope 51.50 mV decade^{-1}) and for the same electrode after regeneration (slope 53.78 mV decade^{-1}).

It was found that the lifespan of the regenerated electrode was limited to 4 h due to the ease of leaching of the lipophilic salts from the gel layer at the electrode surface compared with those that were attached homogeneously to the PVC network through the solvent mediator.

With regard to EZ-PTS and EZ-PMO, they were not affected by soaking for intervals reaching 24 h. This can be attributed to the high rigidity of the membranes which prevents the penetration of anions into external surface during the regeneration process.

3.5. Effect of pH

The effect of pH using 1×10^{-3} mol L^{-1} of ezetimibe solution on the electrode(s) potential was investigated. The solution was acidified by the addition of very small volumes of 0.1 mol L^{-1} hydrochloric acid then the pH value was increased gradually using 0.1 mol L^{-1} sodium hydroxide for each pH value, the potential was recorded and thus the potential-pH curves for ezetimibe concentration were

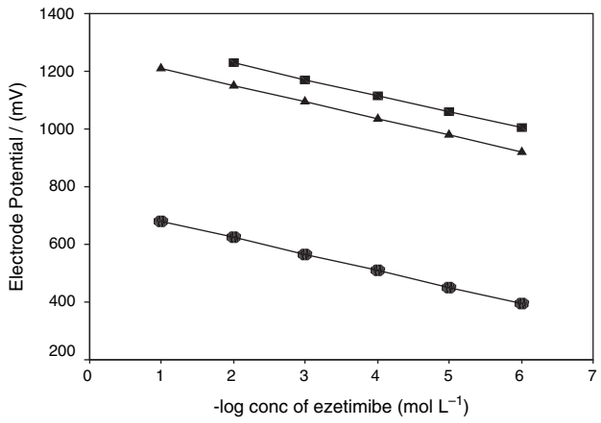

Fig. 2. Typical calibration graph of ezetimibe sensors: (●) EZ-PTS, (■) EZ-PMO and (▲) EZ-PTS/PMO.

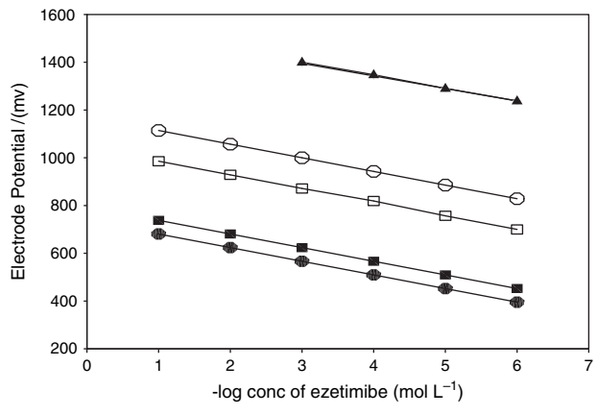

Fig. 3. Calibration graphs obtained at 25 ± 1 °C after soaking the EZ-PTS/PMO electrode for 5 h (●), 24 h (■), 7 days (□), 15 days (○), 28 days (▲).

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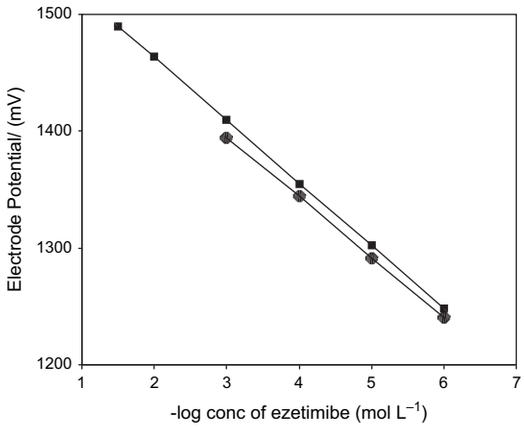

Fig. 4. Regeneration of EZ-PTS/PMO electrode: (●) Calibration graph of an exhausted electrode, (■) calibration graph of electrode after regeneration.

constructed as in Figure 5. It was evident that the electrodes do not respond to pH changes in the range 3–7. The electrode(s) potential is practically independent of pH, and in this range the electrode(s) can be safely used for ezetimibe determination. Below pH 3, the potential of the electrode increased with the increase of analyte acidity which may be ascribed to extraction of H^+ ions by membrane. While at pH more than 7, the response of the electrode decreased which may be attributed to increase of OH^- concentration.[21]

3.6. Selectivity of the Electrodes

The influence of some inorganic cations, sugars, amino acids and simvastatin on the ezetimibe electrodes was investigated. The selectivity coefficient $K^{pot}_{EZJ^+}$ of the electrodes as shown in Table III, reflect a very high selectivity

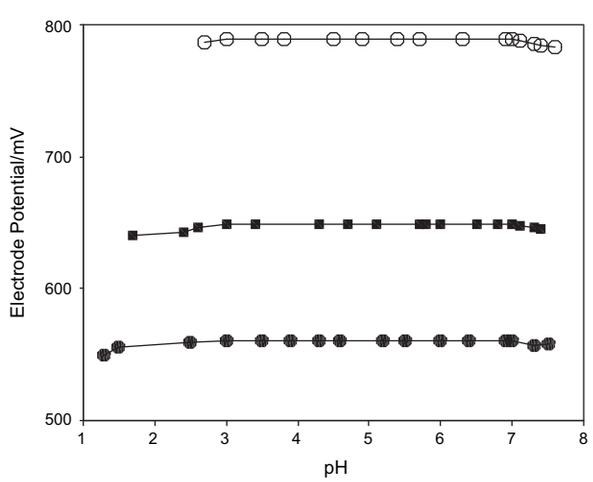

Fig. 5. Effect of pH on electrode potential/mV of ezetimibe sensors using 1×10^{-3} M (●) EZ-PTS, (■) EZ-PMO and (◯) EZ-PTS/PMO coated wire electrode.

Table III. Selectivity coefficients and tolerance values for the ezetimibe responsive electrodes.

	$K^{pot}_{EZ-J^+}$		
Interferent	EZ-PTS	EZ-PMO	EZ-PTS/PMO
Na^+	1.3×10^{-5}	1.0×10^{-3}	3.1×10^{-4}
K^+	3.6×10^{-5}	1.7×10^{-3}	8.1×10^{-3}
NH^+	8.9×10^{-4}	2.5×10^{-3}	5.5×10^{-3}
$Ca2^+$	1.1×10^{-5}	3.8×10^{-4}	1.3×10^{-4}
$Mg2^+$	2.6×10^{-4}	5.8×10^{-4}	7.8×10^{-4}
$Cu2^+$	1.0×10^{-4}	3.4×10^{-4}	4.5×10^{-4}
$CO3^+$	1.4×10^{-4}	1.1×10^{-6}	5.1×10^{-4}
$Fe3^+$	1.8×10^{-4}	1.3×10^{-3}	1.1×10^{-4}
$AL3^+$	7.4×10^{-3}	5.5×10^{-3}	6.7×10^{-4}
Starch	1.1×10^{-5}	6.8×10^{-3}	1.0×10^{-3}
Glucose	5.5×10^{-4}	4.9×10^{-4}	8.5×10^{-4}
Lactose	1.3×10^{-5}	1.9×10^{-5}	1.4×10^{-3}
L-Histadine	2.4×10^{-5}	6.6×10^{-4}	2.4×10^{-3}
DL-Serine	1.9×10^{-5}	3.2×10^{-4}	4.2×10^{-4}
Ornithine	7.2×10^{-4}	4.3×10^{-4}	1.8×10^{-4}
Glycine	7.7×10^{-3}	1.8×10^{-4}	6.2×10^{-4}
L-Cystine	8.0×10^{-3}	2.3×10^{-3}	1.4×10^{-3}
L-Valine	5.1×10^{-3}	2.8×10^{-3}	3.0×10^{-3}
L-Adenine	4.8×10^{-4}	5.3×10^{-3}	8.8×10^{-4}
Simvastatine	8.6×10^{-3}	9.2×10^{-4}	6.9×10^{-3}

of the investigated electrodes for ezetimibe. The mechanism of selectivity was mainly based on the stereospecificity and electrostatic environment, and is not dependent on the matching between the locations of the lipophilic sites in the competing species in the bathing solution side and those present in the receptor of the ion-exchanger. The inorganic cations do not interfere because of differences in the inorganic particle size, mobility and permeability. Also, the smaller the energy of hydration of cation, the greater the response of the membrane. As shown in Table III, the electrodes exhibit good tolerance towards sugars, amino acids and urea.

3.7. Analytical Applications

The investigated electrodes were found to be useful in the potentiometric determination of ezetimibe in pure solutions by calibration graph and standard addition method. The ezitimibe-containing pharmaceutical preparation (Ezetrol®10 mg/tablet) has been assayed using the investigated electrodes. The results obtained were in good agreement with those obtained from the reported method.[22] The latter depends on the spectrophotometric determination of ezetimibe using Fe (III) chloride in the presence of 0.1 mol L^{-1} hydrochloric acid at 740 nm. The results were summarized in Tables IV–VI.

3.7.1. Content Uniformity Assay of Ezetrol® (10 mg/tablet)

The proposed electrodes described good accuracy and precision for the quality control tests, the content uniformity

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Table IV. Statistical treatment of the data obtained for the determination of ezetimibe in pure form by the proposed and reported method.[22]

Calibration method									
EZ-PTS			EZ-PMO			EZ-PTS/PMO			Reported method
Conc. taken (mol L^{-1})	Found-log conc. (mol L^{-1})	Recovery (%)	Conc. taken (mol L^{-1})	Found-log conc. (mol L^{-1})	Recovery (%)	Conc. taken (mol L^{-1})	Found-log conc. (mol L^{-1})	Recovery (%)	Recovery (%)
1.0×10^{-6}	5.99	99.83	1.0×10^{-6}	5.94	99.00	1.0×10^{-6}	5.96	99.33	99.47
5.0×10^{-6}	5.27	99.42	5.0×10^{-6}	5.26	99.23	1.0×10^{-5}	4.93	98.60	98.00
1.0×10^{-5}	4.96	99.20	1.0×10^{-5}	4.97	99.40	1.0×10^{-4}	3.98	99.50	99.00
5.0×10^{-5}	4.24	98.58	5.0×10^{-5}	4.25	98.81	1.0×10^{-3}	2.99	99.67	98.50
1.0×10^{-4}	3.94	98.50	1.0×10^{-4}	3.96	99.00	1.0×10^{-2}	2.00	100.00	99.60
5.0×10^{-4}	3.29	99.67	5.0×10^{-4}	3.28	99.36	1.0×10^{-1}	0.99	99.00	98.67
1.0×10^{-3}	2.97	99.00							98.62
5.0×10^{-3}	2.26	98.22							
Mean ± SD	99.05 ± 0.38			99.13 ± 0.23			99.35		98.84
n	8			6			6		7
Variance	0.34			0.05			0.25		0.56
SE**	0.21			0.01			0.20		0.20
%RSD	0.59			0.24			0.50		0.57
t-test	0.735 (2.145)*			1.315 (2.179)*			1.315 (2.179)*		
F-test	1.07 (3.79)*			5.85 (7.46)*			5.85 (7.46)*		

Notes. *The figures in parentheses are the tabulated *t*- and *F*-tests at $p = 0.05$;[26] **SE (% Error) = %RSD/$\sqrt{n}$.

assay showed that the mean recoveries ± standard deviation were 99.33 ± 0.819, 99.28 ± 0.576 and 99.39 ± 0.411 for EZ-PTS, EZ-PMO and EZ-PTS/PMO, respectively.

3.7.2. Application to Biological Fluids

Ezetimibe is primarily metabolized in the small intestine and liver via glucuroide conjugation with subsequent biliary and renal excretion. In humans, ezetimibe is rapidly metabolized to ezetimibe-glucuronide. Ezetimibe and ezetimibe-glucuronide are the major drug-derived compounds detected in plasma, constituting approximately 10 to 20% and 80–90% of the total drug in plasma, respectively. Both ezetimibe and ezetimibe-glucuronide are slowly eliminated from plasma with a half-life of approximately 22 h for both ezetimibe and ezetimibe-glucuronide. Following oral administration of 14 C-ezetimibe (20 mg) to human subjects, total ezetimibe (ezetimibe and ezetimibe-glucuronide) accounted for approximately 93% of the total radioactivity in plasma. After 48 h, there were no detectable levels of radioactivity in the plasma. While the ezetimibe-glucuronide was

Table V. Statistical treatment of the data obtained for the determination of ezetimibe in dosage form by the proposed and reported method[22] "Calibration method."

Calibration method									
EZ-PTS			EZ-PMO			EZ-PTS/PMO			Reported method
Conc. taken (mol L^{-1})	Found-log conc. (mol L^{-1})	Recovery (%)	Conc. taken (mol L^{-1})	Found-log conc. (mol L^{-1})	Recovery (%)	Conc. taken (mol L^{-1})	Found-log conc. (mol L^{-1})	Recovery (%)	Recovery (%)
5.0×10^{-6}	5.27	99.42	5.0×10^{-6}	5.22	98.47	5.0×10^{-6}	5.28	99.60	97.00
1.0×10^{-5}	4.94	98.80	1.0×10^{-5}	4.92	98.40	1.0×10^{-5}	4.98	99.60	99.00
5.0×10^{-5}	4.22	98.12	5.0×10^{-5}	4.24	98.58	5.0×10^{-5}	4.26	99.05	98.00
1.0×10^{-4}	3.95	98.75	1.0×10^{-4}	3.96	99.00	1.0×10^{-4}	3.97	99.25	97.50
5.0×10^{-4}	3.27	99.06	5.0×10^{-4}	3.26	98.76	5.0×10^{-4}	3.26	98.76	98.20
1.0×10^{-3}	2.94	98.00	1.0×10^{-3}	2.99	99.67	1.0×10^{-3}	2.94	98.00	99.67
									98.86
Mean±SD	98.69 ± 0.55			98.81 ± 0.47			99.04		98.32
n	6			6			6		7
Variance	0.29			0.22			0.37		0.85
SE**	0.22			0.19			0.25		0.32
%RSD	0.55			0.48			0.61		0.94
t-test	0.949 (2.201)*			1.305 (2.201)*			1.776 (2.201)*		
F-test	2.87 (4.39)*			3.82 (4.39)*			2.33 (4.39)*		

Notes. *The figures in parentheses are the tabulated *t*- and *F*-tests at $p = 0.05$;[26] **SE (% Error) = %RSD/$\sqrt{n}$.

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Table VI. Statistical treatment of the data obtained for the determination of ezetimibe in dosage form by the proposed and reported method[22] "Standard addition method."

Standard addition method										
EZ-PTS			EZ-PMO			EZ-PTS/PMO			Reported method	
Conc. taken (mol L^{-1})	Found-log conc. (mol L^{-1})	Recovery (%)	Conc. taken (mol L^{-1})	Found-log conc. (mol L^{-1})	Recovery (%)	Conc. taken (mol L^{-1})	Found-log conc. (mol L^{-1})	Recovery (%)	Recovery (%)	
9.0×10^{-6}	4.99	98.89	9.0×10^{-6}	4.97	98.49	5.0×10^{-6}	5.00	99.88	97.00	
1.0×10^{-5}	4.93	98.60	1.0×10^{-5}	4.90	98.00	1.0×10^{-5}	4.90	98.00	99.00	
5.0×10^{-5}	4.21	97.88	5.0×10^{-5}	4.20	97.65	5.0×10^{-5}	4.24	98.58	98.00	
9.0×10^{-5}	4.03	99.61	9.0×10^{-5}	4.00	98.87	1.0×10^{-4}	4.01	99.12	97.50	
1.0×10^{-4}	3.96	99.00	1.0×10^{-4}	5.94	98.50	5.0×10^{-4}	3.96	99.00	98.20	
5.0×10^{-4}	3.26	98.76	5.0×10^{-4}	3.21	97.24	1.0×10^{-3}	3.28	99.36	99.67	
									98.32	
Mean±SD	98.79 ± 0.56			99.13 ± 0.61			98.99		98.32	
n	6			6			6		7	
Variance	0.32			0.37			0.42		0.85	0.32
SE**	0.23			0.25			0.26		0.32	0.94
%RSD	0.57			0.62			0.65		0.94	
t-test	1.187 (2.201)*			0.467 (2.201)*			1.609 (2.201)*			
F-test	2.67 (4.39)*			2.31 (4.39)*			2.03 (4.39)*			

Notes. *The figures in parentheses are the tabulated *t*- and *F*-tests at $p = 0.05$[26]; **SE (% Error) = %RSD/$\sqrt{n}$.

Table VII. Determination of ezetimibe in spiked human serum and urine using ezetimibe electrodes.

	EZ-PTS		EZ-PMO		EZ-OPT/PMO	
Statistical parameters	Urine solution	Serum solution	Urine solution	Serum solution	Urine solution	Serum solution
Mean	98.01	98.99	98.66	98.49	98.73	98.99
n	7	7	6	7	6	6
Variance	0.29	0.34	0.39	0.23	0.18	0.22
SE**	0.54	0.22	0.25	0.18	0.17	0.21
%RSD	0.20	0.59	0.64	0.48	0.43	0.48

Note. **SE (% Error) = %RSD/$\sqrt{n}$.

the major component in urine and accounted for 9% of the administrated dose. Therefore this method is useful for spiked urine and serum but clearly unadequate for real biological fluid's analysis.[23] The results obtained for determination of ezetimibe in spiked human serum and urine were summarized in Table VII.

4. VALIDATION OF THE PROPOSED ION-SELECTIVE ELECTRODE METHOD

4.1. Linearity

Under the optimal experimental ion selective electrode conditions, linear relationships exist between the electrodes potential/mV and the logarithm of corresponding concentration of the investigated drug. The regression data, correlation coefficients (r) and other statistical parameters were listed in Table II.

4.2. Detection Limit

The detection limit of the investigated drug was calculated according to IUPAC recommendations[24] 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 values were 2.5×10^{-7}, 5.0×10^{-7} and 1.9×10^{-7} mol L^{-1} indicate that the proposed ISE method is sensitive for detection of very small concentrations ($1.0 \times 10^{-6} - 1.0 \times 10^{-1}$, $1.0 \times 10^{-6} - 1.0 \times 10^{-2}$ and $1.0 \times 10^{-6} - 1.0 \times 10^{-1}$ mol L^{-1}) of ezetimibe.

4.3. Robustness and Ruggedness

The robustness of the proposed method was tested by investigating the capacity of the method to remain unaffected by a small but a deliberate variation in method parameters and provides an indication of its reliability during normal usage.[25] The robustness of the proposed method was carried out by using phosphate buffer pH 6 ± 1 and the percentage recoveries were 99.36 ± 0.335, 98.92 ± 0.254 and 99.45 ± 0.478 for the three prepared electrodes, these results were closely in agreement with those obtained from standard drug solutions (Table II). While the ruggedness of the proposed method was investigated by measuring the degree of reproducibility at test results obtained by

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Table VIII. Determination of ezetimibe in spiked placebo samples using ezetimibe electrodes.

Statistical Parameters	EZ-PTS	EZ-PMO	EZ-OPT/PMO
Mean	99.35	99.18	99.41
n	9	9	9
SD	0.36	0.87	0.52
%RSD	0.36	0.88	0.53

the analysis of the same samples under a variety of conditions such as different laboratories, analysts and instruments. Table II, indicates the reproducibility upon using another model of pH-meter (Mettler Toledo pH-meter).

4.4. Accuracy and Selectivity

The selectivity of the proposed ion-selective electrode method was investigated by the determination of ezetimibe in spiked placebo samples prepared from serial concentrations of ezetimibe reference standards. The results summarized in Table VIII, show that the proposed method is an accurate one for the determination of ezetimibe in its pharmaceutical preparation without interfering from the coformulated adjuvants as indicated by the percentage recovery values.

4.5. Precision

The precision of the proposed ion selective electrode method, measured as percentage relative standard deviation (%RDS) was tested by repeating the proposed method for determination of the investigated drug in its pharmaceutical preparation to nine replicates. The %RSD values for the repeated determinations were 0.488%, 0.476% and 0.255% for determination of ezetimibe in Ezetrol® (10 mg/tablet) using EZ-PTS, EZ-PMO and EZ-PTS/PMO electrodes, respectively. The above %RSD values are less than 2% indicating good precision.[26]

5. CONCLUSION

The proposed potentiometric method based on the construction of different types of electrodes with individual and mixed ion exchangers might be useful analytical tool for the determination of ezetimibe in different samples. The present electrodes show high sensitivity, reasonable selectivity, fast static response, long term stability and applicability over a wide concentration range. The proposed procedures were also highly selective, since no interferences from the excepients, impurities, or other accompanying drug components and matrix components in biological fluids.

Acknowledgments: The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGP-VPP-030. Thanks also for Miss. Fawzeiah Al-Miman for providing the polymers.

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