ELECTROCHEMICAL SENSORS FOR DIRECT DETERMINATION OF SIMVASTATIN IN PHARMACEUTICAL FORMULATIONS AND BIOLOGICAL FLUIDS

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ABSTRACT

The construction and performance characteristics of simvastatin (SIM) selective electrodes were developed. Three types of electrodes: plastic membrane I, coated wire II, and coated graphite rod III were constructed based on the incorporation of simvastatin with phosphotungstic (PTA) or phosphomolybdic (PMA) acids and mixed ion pair (PTA/PMA) for the three electrodes, respectively. The influence of membrane composition, kind of plasticizer, type of ion-pair, pH of the test solution, soaking time, and foreign ions on the electrodes was investigated. The electrodes showed a Nernstain response with a mean calibration graph slope of 56.24±0.43, 55.44±0.14 and 58.93±0.34 mV decade^{-1} at 25ºC over simvastatin concentration range from 1.0 x 10^{-6}-5.0 x 10^{-2}, 9.0 x 10^{-6}-5.0 x 10^{-3} and 9.0 x 10^{-7}-1.0 x 10^{-2} mol L^{-1}, with detection limit of 5.0 x 10^{-7}, 3.9 x 10^{-6} and 3.2 x 10^{-7} mol L^{-1} for electrode I, II and III, respectively. The pH range for the proposed electrodes was 4-7. The influence of possible interfering species such as common inorganic cations, many sugars, amino acids and a pharmacologically related drug 'ezetimibe' was studied. Statistical student's *t*-test and *F* test showed insignificant systematic error between proposed and official methods.

Keywords: Plastic membrane; Coated wire electrode; Coated graphite rod; Ion-selective electrodes; Simvastatin; Potentiometric determination

INTRODUCTION

Simvastatin (SIM), (Figure 1), butanoic acid, 2,2-dimethyl-,1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl) ethyl]-1-naphtylalenyl ester, [1S-[1a,3a,7b,8b(2S,4S),8ab]]. It belongs to the group of cholesterol-lowering lactones known as statins, which in 2007 have been among the most widely prescribed drugs in the world. Statins lower cholesterol by inhibiting the synthesis of mevalonic acid, which is a key precursor in cholesterol synthesis. Dropping mevalonic acid levels triggers the expression of more low-density lipoprotein (LDL) receptors in the liver, which then removes LDL from the blood stream [1].

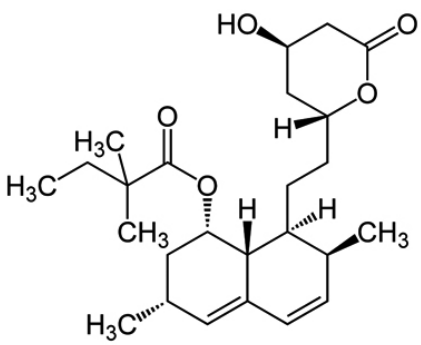

Figure 1: Chemical Structure of Simvastatin.

SIM is the subject of monograph in the USP XXX [2]. HPLC/UV detection at 238 nm was recommended for its determination in raw material and in tablets. The most widely used techniques for the assay of SIM in dosage forms or biological fluids were high performance liquid chromatography [3-5], liquid chromatography-mass spectrometry [6, 7], gas chromatography-mass spectrometry [8], spectrophotometry [9], colorimetry [10] and voltammetry [11].

Electroanalytical techniques have been developed as inexpensive and simple analytical methods with remarkable detection sensitivity, reproducibility and accuracy. These techniques can be classified into three main categories potentiomerty, coulometry and voltammetry [12-14].

Although potentiomerty has some advantages over other techniques being easy, precise, accurate and of low expense, no SIM ion-selective electrode has been constructed yet. In the present study new selective membrane electrodes, of three types: plastic membrane, coated wire and coated graphite electrodes have been constructed for the determination of SIM in pure form, pharmaceutical preparations and biological fluids.

EXPERIMENTAL

2.1. Instrumentation

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.

2.2. Reagents and Materials

All chemicals used were of analytical grade, pure grade simvastatin (SIM) was kindly supplied from Saudi Pharmaceutical Industries and Medical Appliances Corporation, Al-Qassim Pharmaceutical Plant (SPIMACO) Saudi Arabia. The pharmaceutical preparation (Zocor® 10 mg/tablet) was provided by Merck Sharp & Dohme Limited, USA. Methanol 99.0 %, diethyl ether 99.9%, Acetone 99.9%, di-butyl phthalate (DBP) 99.0 % and tetrahydrofuran (THF) 97.0% were provided by Fluka, Switzerland. Poly (vinyl chloride) (PVC) high molecular weight and phosphotungstic acid 99.1 % were purchased from Aldrich, Germany. Urine samples were obtained from healthy volunteers and serum samples (Multi-Serum Normal, Ranbdox Laboratories UK) were obtained from commercial sources.

2.3. Standard Drug Solution

Stock SIM solution 0.1 mol L^{-1} was prepared daily by dissolving 1.046 g of drug in 25 mL methanol. Working solutions were prepared by appropriate dilution with distilled water.

2.4. Preparation of Simvastatin Ion-pair

The ion-pair was prepared by mixing 150 mL of SIM 1.0 x 10^{-2} mol L^{-1} and 50 mL of 1.0 x 10^{-2} mol L^{-1} phosphotungstic acid or phosphomolybdic acid. The resulting precipitates were filtered, washed thoroughly with distilled water and air dried. The chemical composition of precipitates was found to be [SIM-PTA] and [SIM-PMA] as confirmed by (C, H, O) elemental analysis at King Saud University, Analysis Center, results are given in Table 1.

2.5. Membrane Composition

The membrane composition was studied by varying the percentages (w/w) of the ion pair, poly (vinyl chloride) PVC and plasticizer (DBP), until the optimum composition that exhibits the best performance characteristics was obtained. The membranes were prepared by dissolving the required amount of the ion-pair, PVC and (DBP), in 5 mL tetrahydrofuran (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.

2.6. Electrode Construction

2.6.1. Plastic membrane electrode:

A circular membrane was attached to a poly-ethylene tube (8 mm diame-

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ter) in electrode configuration by means of PVC-THF solution. A mixture containing equal volume of 1.0 x 10^{-3} SIM and 1.0 x 10^{-3} mol L^{-1} potassium chloride was used as internal reference solution in which the Ag/AgCl reference electrode was dipped. The constructed electrode was pre-conditioned after preparation by soaking for at least 24 h in 1.0 x 10^{-3} mol L^{-1} SIM 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 // SCE.

Table 1: Elemental analysis of the simvastatin ion-pairs.

Ion-associate	C%		H%		N%	
	Found	Calculated	Found	Calculated	Found	Calculated
I[a]	21.75	21.78	2.80	2.78	21.33	21.29
II[b]	25.05	28.08	3.60	3.58	31.47	31.44
I[a] $[C_{25}H_{38}O_5]_3[P(W_3O_{10})_4$ **II**[b] $[C_{25}H_{38}O_5]_3[PO_4.12MoO_2]$						

2.6.2. Coated wire electrode:

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. The coating solution was described under (2.5. membrane composition). 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 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 formed [15]. The prepared electrode was conditioned by soaking for 4 h in 1.0 x 10^{-3} mol L^{-1} SIM solution. All potentiometric measurements were performed using the following cell assembly: Al / membrane / test solution // KCl salt bridge // SCE.

2.6.3. Coated graphite electrode:

A pure graphite rod 4.0 cm length and 4.0 mm diameter was insulated by tight polyethylene tube, leaving 2.0 cm at one end for coating and 1.0 cm at the other end for connection. The polished electrode surface was coated with the active membrane by dipping the exposed end into the coating solution that was described under (2.5. membrane composition), ten times and allowing the film left on graphite rod to dry in air for 1 min each time. The prepared electrode was preconditioned by soaking for 6 h in 1.0 x 10^{-3} mol L^{-1} SIM solution [16]. All potentiometric measurements were performed using the following cell assembly: Graphite rode / membrane / test solution // KCl salt bridge // SCE.

2.7. Electrode Calibration

Ten mL aliquots of 1.0 x 10^{-1}-1.0 x 10^{-7} mol L^{-1} standard solutions were transferred into 50 mL beaker and the electrode(s) in conjunction with double junction Ag/AgCl reference electrode were immersed in the solution. The measured potential was plotted against the logarithm of drug concentration. The electrode(s) was washed with distilled water and dried with tissue paper between measurements.

2.8. Electrode Selectivity

Selectivity coefficients $K^{Pot}_{SIM,J}{}^{z+}$ of the electrodes towards different cationic species were determined by the separate solution method [17] in which the following equation was applied.

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

Where, E_1 is the electrode potential in 1.0 x 10^{-3} mol L^{-1} SIM 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 some cases, when the selectivity coefficients were not very high, mixed solution method was applied [18].

2.9. Effect of pH

The effect of pH of the test solution 1.0 x 10^{-3} mol L^{-1} on the electrode(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.

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 SIM-electrode(s) with the double junction Ag/AgCl reference electrode was connected to the second instrument. Thirty mL aliquots of 1.0 x 10^{-3} mol L^{-1} drug solution were transferred to a 100 mL beaker where the electrodes were immersed. The potential readings corresponding to different pH values were recorded and plotted.

2.10. Standard Addition Method

The fabricated electrode(s) was immersed into sample of 50 mL with unknown concentration (ca. 1.0 x 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 obtained. One can determine the concentration of the testing sample from the change of potential ΔE (E_2-E_1). The standard addition technique was used for the analysis of SIM tablets [19].

2.11. Analytical Applications

2.11.1. Determination of Simvastatin in Tablets

Ten tablets Zocor® (10 mg/tablet) were finely powdered and an accurately weighed amount of the powder equivalent to 10 mg of (SIM) was transferred into a small conical flask. Extract with 3x30 mL portion of methanol was performed. The contents were then filtered into a 100-mL standard flask and diluted to the mark with methanol. Working solutions were prepared in the range of 1.0 x 10^{-5}- 5.0 x 10^{-3} mol L^{-1}, by appropriate dilution with distilled water. Direct determination and standard addition method were applied using SIM-electrodes.

2.11.2. Content Uniformity Assay of Simvastatin Tablets

Ten individual tablets of Zocor® (10 mg/tablet) were placed each separately in 100 mL measuring flask and dissolved in 50 mL methanol then complete to volume with distilled water. The electrode(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.

2.11.3. Application to Serum and Urine

An aliquot of standard methanolic solution of SIM containing 10.0 mg was added to 5 mL of serum or urine sample in a 100-mL measuring flask and mixed for 1 min. Then, the spiked urine sample was completed to volume using distilled water. For spiked serum sample 10.0 mL of diethyl ether was added 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. Working solutions for both urine and serum were prepared in the range of 1.0 x 10^{-5}- 1.0 x 10^{-2} mol L^{-1}, by appropriate dilution with distilled water and subsequently analyzed according to general analytical procedures.

RESULTS AND DISCUSSION

3.1. Optimization of Membrane Composition

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 [20, 21]. Also, the amount of plasticizer should be suitable for good physical properties and at the same time efficiently acts as a solvent mediator. 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 depending on the properties of both the ion-pair and the matrix [22]. In this work the ratio of plasticizer (DBP) to polymer was kept constant at 1:1, while the amount of ion- pair was varied.

Several compositions for the electrodes were investigated in which the ion-pair percentage ranged from 5-15% for SIM-PTA and SIM-PMA and from 5-20% for SIM-PTA/PMA of each ion-pair. The preparation process was highly reproducible as revealed by the low relative standard deviations values

of the slopes obtained employing the prepared membranes (% RSD was about 0.43%, 0.14% and 0.34%) for the above three mentioned electrodes, respectively.

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

3.2. Nature and Response Characteristics of the Sensors

The critical response characteristics of plastic membrane, coated wire, and coated graphite rod-electrodes were determined and the results were summarized in Table 2. The electrode(s) exhibits a Nernstain response over the concentration range from 1.0 x 10^{-6}-9.0 x 10^{-2}, 9.0 x 10^{-6}-5.0 x 10^{-3} and 9.0 x 10^{-7}-1.0 x 10^{-2} mol L^{-1} SIM for electrode I, II and III, respectively, with slopes of 56.24±0.43, 55.44±0.14 and 58.93±0.34 mV $decade^{-1}$ change in concentration for electrode I, II and III, respectively as in Figure 2. The choice of 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 the ion-pair complex. The response time of the electrode(s) was tested for 1.0x10^{-7}-1.0x10^{-1} mol L^{-1} SIM solutions. The electrode(s) exhibits a fast dynamic response of 30, 25 and 20 s for a period of 25, 30 and 35 days for electrode I, II and III respectively, without significant change in the electrode(s) parameters.

Table 2: Critical response characteristics of simvastatin sensors.

Parameter[a]	SIM-plastic membrane sensor	SIM-coated wire sensor	SIM-coated graphite sensor
Slope (mV $decade^{-1}$)	56.24±0.43	55.44±0.14	58.93±0.34
Intercept	422.70	336.84	449.91
Correlation coefficient r.	0.9997	0.9998	0.9999
Linear range (mol L^{-1})	1.0x10^{-6}-5.0x10^{-2}	9.0x10^{-6}-5.0x10^{-3}	9.0x10^{-7}-1.0x10^{-2}
LOD (mol L^{-1})	5.0 x10^{-7}	3.9 x10^{-6}	3.2 x10^{-7}
Response time (s)	30	25	20
Working pH range	4-7	4-7	4-7
Lifetime /day	25	30	35
Accuracy (%)	99.52	99.17	99.48
Standard deviation	0.3	0.6	0.8
Robustness[b]	99.54±0.27	99.46±0.36	99.76±0.56
Ruggedness[c]	99.41±0.39	99.03±0.62	99.79±0.45

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

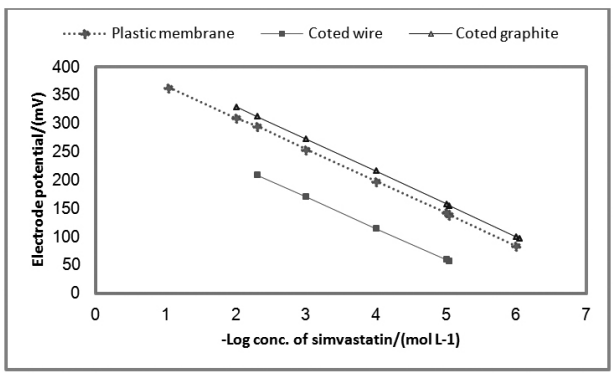

Figure 2: Typical Calibration Graphs of Simvastatin Sensors.

3.3. Effect of Soaking time and Regeneration of the Electrode

The performance characteristics of SIM electrode(s) was studied as a function of soaking time. For this purpose the electrode(s) was soaked in 1.0 x 10^{-3} mol L^{-1} solution of SIM and the calibration graphs were plotted after optimum soaking time 24, 4, and 6 h for electrode I, II and III, respectively. The slope of the calibration curve was 56.24±0.43, 55.44±0.14 and 58.93±0.34 mV $decade^{-1}$, at 25 ºC for electrode I, II and III respectively. The electrode(s) was continuously soaked on 1x10^{-3} mol L^{-1} solution of SIM for 7, 12, 20, 25 and 35 days. The calibration plot slopes decreased slightly to 54.70, 53.91 and 56.29 mV $decade^{-1}$ after 20 days for electrode I, II and III respectively, and continued to decrease reaching 51.60, 48.91 and 52.60 mV $decade^{-1}$ after 35 days. This reveals that soaking of the electrode(s) in the drug solution for a long time has a negative effect on the response of the membrane. The same effect appears after working with the electrode(s) for a long time. The regeneration of the electrode(s) was tried simply by reformation of the ion-pair on the external gel layer of membrane [23]. The regeneration of the SIM membrane was successfully achieved by soaking the exhausted electrode(s) for 24 h in a solution that was 1.0 x 10^{-2} mol L^{-1}phophotungstic or phosphomolybdic acid, followed by soaking for 3 h in 1.0 x 10^{-2} mol L^{-1} SIM solution. Figures 3-5, show the calibration graphs for an exhausted electrode(s) (slopes 51.60, 48.91 and 52.60 mV $decade^{-1}$) for electrode I, II and III respectively, and for the same electrode(s) after regeneration (slopes 52.40, 52.49 and 53.53 mV $decade^{-1}$). It was found that the lifespan of the regenerated electrode(s) is limited to 6 h due to the ease of leaching of the lipophilic salts from the gel layer at the electrode(s) surface compared with those that are attached homogeneously to the PVC network through the solvent mediator.

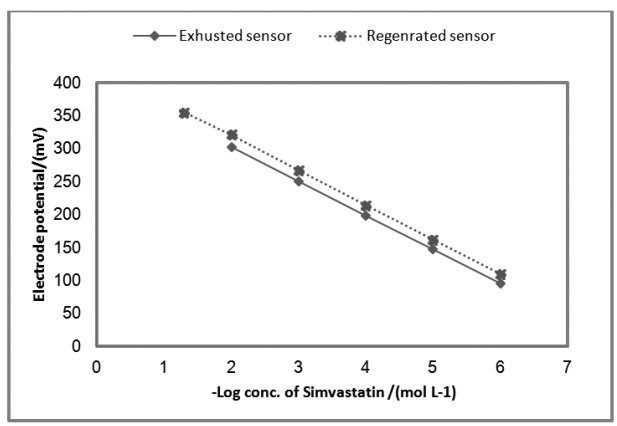

Figure 3: Regeneration of SIM-PTA Plastic Membrane Sensor

3.4. Effect of pH

The effect of pH of the SIM solutions (1.0 x 10^{-3} mol L^{-1}) on the electrode(s) potential was investigated. The solutions were acidified by the addition of very small volumes of 0.1 mol L^{-1} hydrochloric acid then the pH value was increa-

sing gradually using 0.1 mol L^{-1} sodium hydroxide. The potential was recorded for each pH value and then the potential-pH curves for SIM were constructed as in Figure 6. It was found that within the pH range 4-7, the electrode(s) potential is practically independent of pH, and in this range the electrode can be safely used for SIM determination. Below pH 4, 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 [24].

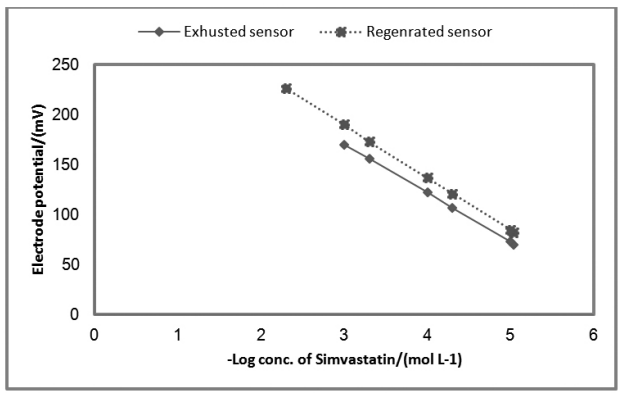

Figure 4: Regenration of SIM-PMA Coated Wire Sensor.

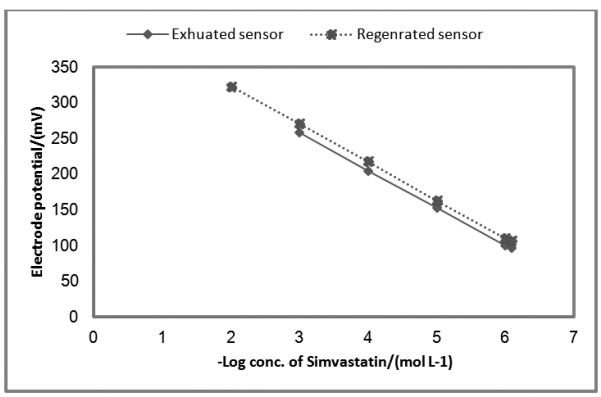

Figure 5: Regeneration of SIM-PTA/PMA Coated Graphite Sensor.

3.5. Selectivity of the Electrode

The influence of some inorganic cations, sugars and amino acids on SIM electrodes was investigated. The results obtained (Table 3) reflect a very high selectivity of the investigated electrodes for SIM. 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+}, Al^{3+} and Fe^{3+} did not interfere because of differences in the inorganic particle size, mobility and permeability. Also as shown in Table 3, the electrodes exhibit good tolerance towards sugars, amino acids and urea. The interference of ezetimibe as co formulated drug with SIM was investigated and the electrodes showed insignificant interferent effect during the determination of SIM.

3.6. Quantification of Simvastatin

Direct potentiometric determination of SIM using SIM electrode(s) type I, II and III, was performed and calculated from the calibration curve or regression equation. The direct potentiometric determination of simvastatin in pure form using the proposed electrodes gave mean recovery% of 99.57±0.53, 99.64±0.37 and 99.69±0.33 for electrode I, II and III, respectively. Furthermore, the results obtained were encouraging so the proposed method was applied for the determination of simvastatin in its pharmaceutical preparations. The results were compared with the HPLC official method [3], and the results are listed in Tables 4-6.

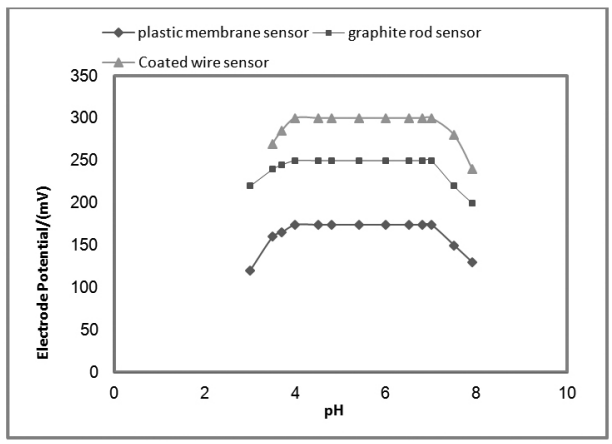

Figure 6: Effect of pH on the Simvastatin Electrodes Potential.

3.7. Method Validation

The method was validated for linearity, accuracy, intra-day and inter-day precision, repeatability, robustness and ruggedness accordance with ICH guidelines [25].

Linear relationships exist between the electrode potential/mV and the logarithm of corresponding concentration of the investigated drug over the range cited in Table 2. The proposed ISE method is sensitive for detection of very small concentrations of SIM (5.0 x 10^{-7}, 3.9 x 10^{-6} and 3.2 x 10^{-7} mol L^{-1}) for electrodes I, II and III, respectively.

The robustness of the proposed method was carried out by using phosphate buffer pH 6±1 and the percentage recoveries were 99.54± 0.27, 99.46±0.36 and 99.76±0.56 for the three prepared electrodes, these results were closely in agreement with those obtained from standard drug solutions, (Table 2). The reproducibility upon using another model of pH-meter (Jenway 3510) was indicated by the results obtained (Table 2).

Also, the proposed method is an accurate one for the determination of SIM in its pharmaceutical preparations without interfering from the coformulated adjuvants as indicated by the percentage recoveries value of (99.52±0.34, 99.17±0.62 and 99.46±0.36). The precision of the method was calculated in terms of (intra-day and inter-day). The RSD % values of intra-day and inter-day studies for the repeated determinations were 0.28 %, 0.16% and 0.32% for determination of simvastatin in Zocor® 10 mg /tablets using electrode I, II and III respectively. The above RSD% values are less than 2% indicating good precision.

3.8. The Electrode Response in Pharmaceuticals

The use of SIM drug in various clinical fields has necessitated an accurate, rapid and quantitative analysis in various matrices (dosage forms and biological fluids). This work proposed a fast, simple, easy, sensitive and straightforward potentiometric method to determine SIM in dosage forms without the need for prior separation and preconcentration or derivatization procedures. The potential of the simvastatin sensors showed no significant difference of response time between aqueous solution of pure drug and its solutions from pharmaceutical preparations. The proposed method described good accuracy and precision for the quality control tests, the content uniformity assay showed that the (RSD < 2%), with mean recoveries and standard deviation of 99.44±0.23, 98.99±0.72 and 99.72±0.19 for electrode I, II and III respectively.

3.9. The Electrode Response in Biological Fluids

As reported earlier [27], following an oral dose of 14C-labeled SIM (40 mg SIM) in man, 13% of the dose was excreted in urine and 60% in feces. Plasma concentrations of total radioactivity (SIM plus 14C-metabolites) peaked at 4 hours and declined rapidly to about 10% of peak by 12 hours post dose. The validity of the proposed method was tested by analyzing SIM in spiked biological fluids. The results were listed in Table 7. The potential of the SIM sensors showed no significant difference of response time between aqueous solution of pure drug and its spiked biological fluids.

Table 3: Selectivity coefficient and tolerance values for simvastatin sensors.

Interferent	SIM-PTA $-\log K^{pot}_{SIM,J}{}^{z+}$		SIM-PMA $-\log K^{pot}_{SIM,J}{}^{z+}$		SIM-PTA/PMA $-\log K^{pot}_{SIM,J}{}^{z+}$	
	SSM[a]	MSM[b]	SSM[a]	MSM[b]	SSM[a]	MSM[b]
Na^+	2.82	2.98	3.11	3.25	3.32	3.42
K^+	2.36	2.42	3.74	---	2.95	3.21
NH_4^+	2.64	2.85	3.64	---	2.74	3.48
Ca^{2+}	3.13	3.24	3.83	---	4.12	---
Mg^{2+}	3.22	3.55	3.35	3.48	4.76	---
Cd^{2+}	3.51	3.78	3.62	---	4.23	4.33
Al^{3+}	3.48	3.65	3.22	3.34	4.33	4.52
Fe^{3+}	3.39	3.58	3.47	3.52	4.68	4.81
L- Valine	---	4.12	---	4.01	---	4.99
L-Histadine	---	4.68	---	5.27	---	4.69
L-Cystine	---	5.01	---	5.13	---	5.53
Glycin	---	4.89	---	4.54	---	4.17
Starch	---	4.22	---	5.12	---	5.02
Lactose	---	4.87	---	3.99	---	5.11
Urea	---	5.26	---	5.48	---	4.84
Ezetimibe	---	5.64	---	5.68	---	4.97
[a]Separate solution method			[b]Mixed solution method			

Table 4: Statistical treatment of the data obtained for the determination of simvastatin in pure form by the proposed and official methods [3]

Calibration method							
SIM-PTA (Plastic membrane)			SIM-PMA (Coated wire)		SIM-PTA/PMA (Coated graphite)		Official method
Taken (mol L^{-1})	Found -log conc. (mol L^{-1})	Recovery %	Found -log conc. (mol L^{-1})	Recovery %	Found -log conc. (mol L^{-1})	Recovery %	Recovery %
$1.0x10^{-5}$	4.97	99.40	4.99	99.80	5.00	100.00	100.00
$5.0x10^{-5}$	4.29	99.74	4.26	99.05	4.30	99.98	100.21
$1.0x10^{-4}$	4.01	100.25	4.00	100.00	3.99	99.75	99.75
$5.0x10^{-4}$	3.26	98.76	3.28	99.36	3.29	99.67	99.97
$1.0x10^{-3}$	2.98	99.33	2.99	99.67	2.99	99.67	99.33
$5.0x10^{-3}$	2.30	99.96	2.30	99.96	2.28	99.09	100.39
Mean±SD	99.57±0.53		99.64±0.37		99.69±0.33		
n	6		6		6		99.94±0.37
Variance	0.28		0.14		0.11		6
SE**	0.22		0.29		0.13		0.14
RSD	0.54		0.38		0.34		0.15
t-test	1.39(2.23)*		0.92(2.23)*		1.26(2.23)*		0.38
F-test	2.00(5.05)*		1.00(5.05)*		0.79(5.05)*		

**The Figures in parentheses are the tabulated t- and F- test at p = 0.05[28]*
*** %Error= %RSD/√n*

Table 5: Statistical treatment of the data obtained for the determination of simvastatin in dosage form by the proposed and official methods [3] "Calibration method".

Calibration method							
SIM-PTA (Plastic membrane)			SIM-PMA (Coated wire)		SI-PTA/PMA (Coated graphite)		Official method
Taken (mol L^{-1})	Found -log conc. (mol L^{-1})	Recovery %	Found -log conc. (mol L^{-1})	Recovery %	Found -log conc. (mol L^{-1})	Recovery %	Recovery %
$1.0x10^{-5}$	4.96	99.20	4.99	99.80	4.97	99.40	99.40
$5.0x10^{-5}$	4.30	99.98	4.27	99.28	4.29	99.74	99.74
$1.0x10^{-4}$	3.96	99.00	3.99	99.75	4.01	100.25	99.75
$5.0x10^{-4}$	3.29	99.67	3.27	99.06	3.26	98.76	98.75
$1.0x10^{-3}$	3.00	100.00	2.99	99.67	2.98	99.30	99.67
$5.0x10^{-3}$	2.27	98.65	2.25	97.78	2.30	99.96	99.96
Mean±SD	99.42±0.55		99.22±0.76		99.57±0.53		99.55±0.43
n	6		6		6		6
Variance	0.31		0.59		0.28		0.18
SE**	0.23		0.31		0.22		0.17
RSD	0.56		0.77		0.53		0.44
t-test	0.45 (2.23)*		0.93 (2.23)*		0.07 (2.23)*		
F-test	1.72(5.05)*		3.27 (5.05)*		1.56(5.05)*		

**The Figures in parentheses are the tabulated t- and F- test at p = 0.05[28]*
*** %Error= %RSD/√n*

Table 6: Statistical treatment of the data obtained for the determination of simvastatin in dosage form by the proposed and official method (25) "Standard Addition method"

Standard addition method							
SIM-PTA (Plastic membrane)			SIM-PMA (Coated wire)		SIM-PTA/PMA (Coated graphite)		Official method
Added (mol L^{-1})	Found -log conc. (mol L^{-1})	Recovery %	Found -log conc. (mol L^{-1})	Recovery %	Found -log conc. (mol L^{-1})	Recovery %	Recovery %
$1.0x10^{-5}$	5.00	100.00	4.98	99.60	4.96	99.20	99.40
$5.0x10^{-5}$	4.28	99.51	4.27	99.28	4.30	99.98	99.74
$1.0x10^{-4}$	3.96	99.00	3.99	99.75	4.00	100.00	99.75
$5.0x10^{-4}$	3.26	98.76	3.28	99.36	3.29	99.67	98.75
$1.0x10^{-3}$	2.99	99.67	2.97	99.00	2.98	99.33	99.67
$5.0x10^{-3}$	2.30	99.96	2.29	99.52	2.28	99.09	99.96
Mean±SD	99.48±0.51		99.42±0.26		99.54±0.39		99.55±0.43
n	6		6		6		6
Variance	0.26		0.08		0.15		0.18
SE**	0.21		0.11		0.16		0.17
RSD	0.52		0.27		0.40		0.44
t-test	0.26 (2.23)*		0.64 (2.23)*		0.04(2.23)*		
F-test	1.44 (5.05)*		2.25 (5.05)*		1.20 (5.05)*		

**The Figures in parentheses are the tabulated t- and F- test at p = 0.05[28]*
*** %Error= %RSD/√n*

Table 7: Determination of simvastatin in spiked technique in human serum and urine using simvastatin electrodes.

Statistical parameters	SIM-PTS (plastic membrane)		SIM-PMA (coated wire)		SIM-PTA/PMO (graphite rod)	
	Urine solution	Serum solution	Urine solution	Serum solution	Urine solution	Serum solution
Mean	99.32	99.06	98.84	98.98	99.17	99.12
n	6	6	6	6	6	6
Variance	0.03	0.84	0.08	0.29	0.29	0.15
SE**	0.07	0.37	0.12	0.22	0.19	0.16
% RSD	0.18	0.93	0.29	0.54	048	0.40

*** %Error= %RSD/√n*

CONCLUSION

In the present study, new three constructed sensors were developed for simvastatin determination over a wide range of concentrations. The results obtained show that the constructed sensors displayed faster response time suitable for analytical use in the determination of simvastatin in drug bulk powder, dosage forms and biological fluids. Apart from showing linear response within wide pH and concentration ranges with high accuracy and sensitivity, they also have high selectivity and reproducibility.

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