International Journal of Physical Sciences Vol. 7(9), pp. 1403 - 1411, 23 February, 2012
Available online at http://www.academicjournals.org/IJPS
DOI: 10.5897/IJPS11.1364
ISSN 1992 - 1950 ©2012 Academic Journals

Full Length Research Paper

Electrochemical sensors for direct potentiometric determination of Voriconazole in pharmaceutical dosage forms and biological fluids

Nawal A. Alarfaj* and Maha F. El-Tohamy

Department of Chemistry, College of Science, King Saud University, P.O. Box 22452, Riyadh 11495, Saudi Arabia.

Accepted 24 January, 2012

The compositions and general performance characteristics of four polyvinyl chloride (PVC) membrane sensors respective to Voriconazole (VR) were described. Two of these sensors are based on the use of ion association complexes of VR with phosphotungstic acid (PTA) and phosphomolybdic acid (PMA) as novel electroactive materials dispersed in *o*-nitrophenyloctyl ether (*o*-NPOE) plasticizer on glass assemblies. The third sensor is a coated wire type and based on the ion association incorporation of the VR with tetraphenylborate (TPB). The forth sensor is a graphite electrode type based on ion association with silicotungstic acid (STA). The developed sensors were used for the determination of VR in pure form in its pharmaceutical formulations and in biological fluids. The sensors displayed Nernstain response 57.56 ± 0.16, 58.45 ± 0.54, 56.17 ± 0.28 and 57.09 ± 0.74 mV at 25ºC, over linear concentration ranges from $9.0x10^{-7}$ to $1.0x10^{-2}$, $1.0x10^{-6}$ to $1.0x10^{-2}$, $1.0x10^{-6}$ to $1.0x10^{-2}$ and $5.0x10^{-6}$ to $1.0x10^{-2}$ molL^{-1} for the four mentioned sensors, respectively. The pH does not affect the sensor performances within the pH range of 3 to 8. Acceptable selectivity was obtained for VR against many inorganic cations, sugars and amino acids. Statistical student's *t*-test and *F* test showed insignificant systematic error between proposed and reported methods.

Key words: Voriconazole, potentiometry, ion-selective electrode, graphite sensors, pharmaceutical formulations, biological fluids.

INTRODUCTION

Voriconazole (VR) is a triazole antifungal that is a derivative of fluconazole. Like all azole antifungals, its mechanism of action is the inhibition of a cytochrome P-450-dependent enzyme, 14-á-sterol demethylase that is essential to the synthesis of ergosterol for the fungal cell membrane (Figure 1). This inhibition is more selective for fungal than for mammalian enzyme systems. The accumulation of 14-alphamethyl sterols results in a decrease in ergosterol, which is an essential component of fungal cell wall formation. The resulting cell wall abnormalities are thought to be responsible for VR's antifungal activity. VR was approved by the Food and Drug Administration in May, 2002 for the treatment of invasive aspergillosis and refractory infections of scedosporiumapiospermum and fusarium. Studies have also shown it to be a promising agent for empiric treatment in febrile neutropenia (Pfizer, Inc. 2002). Human pharmacokinetic data for VR have been published by few methods reported for the determination of VR including high performance liquid chromatography (HPLC) (Gu and Li, 2009; Khoschsorur et al., 2005; Kahke et al., 2009; Michael et al., 2008; Pehourcq et al., 2004; Sinubabu et al., 2007; Thieurillat et al., 2010), liquid chromatography coupled with mass spectrometry (Araujo et al., 2007; Xiong et al., 2010) and spectrophotometry (Adams et al., 2006). In the present study, four sensors have been constructed and used for the determination of VR in bulk drug, its pharmaceutical preparations and biological fluids.

*Corresponding author. E-mail: nalarfaj@hotmail.com. Tel: +96614772245.

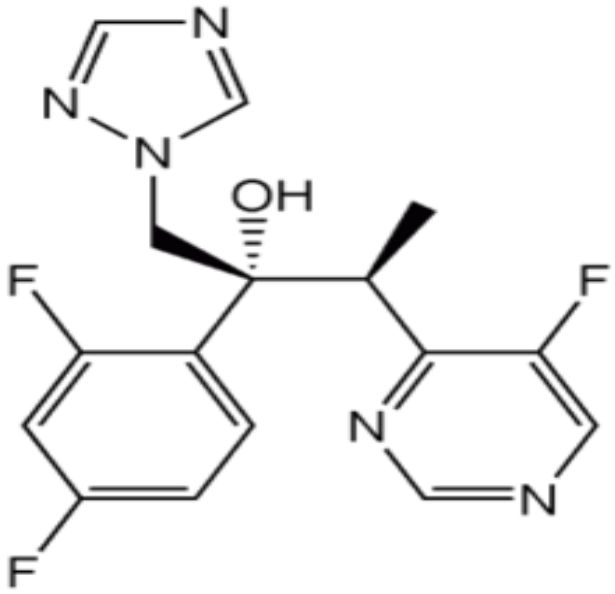

Figure 1. Chemical structure of Voriconazole

METHODOLOGY

Apparatus

The electrochemical measurements were carried out with HANNA instruments pH 211 microprocessor pH-meter. Saturated calomel electrode (SCE) was used as an external reference electrode.

Reagents

All reagents were of analytical grade. Pure grade of VR was kindly supplied by Sigma Co. Polyvinyl chloride (PVC) of high molecular weight was purchased from Aldrich, Germany. Methanol 99.0%, acetone 99.9% and tetrahydrofuran (THF) 97.0% were provided by Fluka, Switzerland. Sodium tetraphenylborate (TPB), phosphotungstic acid (PTA), phosphomolybdic acid (PMA), silicotungstic acid (STA) and o-nitrophenyloctyl ether (*o*-NPOE) were purchased from Aldrich. Carbon rod was obtained from Ultra Carbon Co. (Bay City, Mi, USA). The pharmaceutical dosage forms containing VR were purchased from drug stores. Urine samples were collected from healthy volunteers and serum samples were obtained from blood bank (Zagazig University Hospital).

Standard drug solution

A stock solution 0.1 $molL^{-1}$ was prepared by dissolving 3.493 g of VR in 100 mL methanolic water (50:50 v/v). Serial dilutions ($9.0x10^{-7}$-$1.0x10^{-2}$ $molL^{-1}$) were prepared using distilled water.

Preparation of ion-pairs

The ion-pair VR-phosphotungstate (faint yellow powder), VR-phosphomolybdate (yellowish green crystals), VR-TPB (faint pink powder) and VR-STA (faint yellow powder) were prepared by the addition of 150 mL of 1.0×10^{-2} $molL^{-1}$ VR solution to 50 mL of 1.0×10^{-2} mol L^{-1} of each of the precipitating agents. The precipitates were filtered, washed thoroughly with distilled water and air dried. The chemical composition of the precipitates was found to be VR-PTA, VR-PMA, VR-TPB and VR-STA as confirmed by (C, H, N, O) elemental analysis at Sigma Co. Egypt.

Construction of sensors

Fabrication of the sensors (VR-PTS) or (VR-PMA) and their evaluation were accomplished by the established procedures (Graggs et al., 1974). The master membranes were stored in refrigerator at 4°C. Each fresh membrane sensor was preconditioned by soaking in 1×10^{-3} $molL^{-1}$ VR solution for 24 h.

The coated wire membrane sensor was constructed as pure copper wire of 4.0 cm length and 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 previously under membrane composition. Prior to coating, the polished copper 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 copper 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 was formed. The prepared sensor was conditioned by soaking for 6 h in 1.0×10^{-3}mol L^{-1} VR solution.

The graphite VR-STA was prepared by mixing 190 mg of PVC powder, 10 mg of ion-pair VR-STA and 350 mg of o-NPOE in 5.0 mL of THF. Graphite rode of 5.0 mm in diameter and 15.0 mm long was forced inside polyethylene tube. The end of the graphite rod was washed with acetone, dried in air for 2 to 3 h and coated with the prepared cocktail. The dipping process was repeated 5 to 7 times to make a uniform thin film on the surface of the graphite rod. The solvent was allowed to evaporate in air after each dipping and finally the sensor was left to dry at room temperature for 24 h before use. One drop of mercury was added in the polyethylene sleeve to ensure electrical contact with connection cable. The sensor was conditioned by soaking in 1.0×10^{-3} mol L^{-1} VR solution for 6 h and stored in the same solution when not in use.

Sensors calibrations and selectivities

The preconditioned sensors were calibrated against a double junction Ag/AgCl reference electrode, at 25 ± 1°C, using the direct calibration technique. The sensors were washed with water and dried carefully between measurements. The potential readings were recorded and plotted as a function of logarithm VR concentrations. The selectivity coefficients $K^{Pot}_{VRJ}{}^{z+}$ were determined using separate solution method (SSM) (Ma and Hassan, 1982) in which the following equation is applied:

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

Where $K^{Pot}_{VR.}J^{z+}$ is the selectivity coefficient, E_1 is the electrode potential in $1\times10^{-3}$$molL^{-1}$ VR solution, E_2 is the potential of the electrode in 1×10^{-3} $molL^{-1}$ solution of interference J^{z+} and S is the slope of calibration graph in mV. In some cases, when selectivity coefficients were not very high, mixed solution method (MSM) was used (Moody and Thomas, 1989; Adel et al. 2001).

Determination of Voriconazole in pharmaceutical dosage forms

Determination of Voriconazole in tablets

Ten tablets (Vfend® 50 mg/tablet) were finely powdered and accurate weights were dissolved in 50 mL methanol to obtain sample solutions ranging from 5.0×10^{-6} to 1.0×10^{-3} and 1.0×10^{-5}

Table 1. Elemental analysis of Voriconazole ion-pairs.

Element (%)	VR-PTA[a]		VR-PMA[b]		VR-TPB[c]		VR-STA[d]	
	Found	Calculated	Found	Calculated	Found	Calculated	Found	Calculated
C	14.64	14.68	20.07	20.05	69.46	69.44	14.71	14.69
H	1.09	1.08	1.62	1.58	4.99	4.96	1.14	1.10
N	5.31	5.35	7.34	7.31	10.11	10.13	5.34	5.36
O	17.55	17.53	23.98	23.94	2.36	2.31	17.52	17.54

[a], $[C_{16}H_{14}F_3N_5O]_3[P(W_3O_{10})_4]$; [b], $[C_{16}H_{14}F_3N_5O]_3[PMo_{12}O_{40}]$; [c], $[C_{16}H_{14}F_3N_5O][(C_6H_5)_4$ B Na]; [d], $[C_{16}H_{14}F_3N_5O]_3[H(W_{12}SiO_{40})]$.

to 1.0×10^{-3} molL^{-1} for direct determination and standard addition methods for VR sensors.

Content uniformity assay of Voriconazole tablets: Ten individual tablets of Vfend® 50 mg/tablet were placed in separate 100 mL beaker and dissolved in 50 mL methanol, then completed to volume with distilled water. The electrode(s) was directly immersed into 10 mL of drug sample for five times and then 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.

Application to serum and urine

An aliquot of standard methanolic solution of VR containing 10 mg was added to 5 mL of serum or urine sample in a 100 mL measuring flask and mixed for 1 min. Working solutions were prepared in the range of 1.0×10^{-5} to 1.0×10^{-2} molL^{-1} by appropriate dilution with deionized water and subsequently analyzed according to recommended in the general analytical procedures.

RESULTS AND DISCUSSION

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 (Buck and Linder, 1994; Shoukry et al., 2001; Nour et al. 2000). Also, the amount of plasticizer should be suitable for good physical properties and at the same time efficiently act 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 (Oesh and Simon, 1980). In this work, the ratio of plasticizer (*o*- NPOE) to polymer was kept constant at 1:1, while the amount of pair varied. Several compositions for the electrodes were investigated in which the ion-pair percentage ranged from 5 to 15% for VR-PTA, VR-PMA, VR-TPB and VR-STA. The preparation process was highly reproducible as revealed by the low relative standard deviations (RSD) values of the slopes obtained employing the prepared membranes (RSD was about 0.163, 0.542, 0.284 and 0.735%) for the four mentioned electrodes, respectively. The best performances were obtained using compositions of 10% VR-ion pair, 45% diastolic blood pressure (DBP) and 45% PVC. The optimum compositions were used to prepare membrane electrodes for all further investigations. Results of elemental analysis of membranes are shown in Table 1.

Nature and response characteristics of the sensors

The critical response characteristics of plastic membrane, coated wire and coated graphite rod-electrodes were determined and results are shown in Table 2. The electrode(s) exhibits a Nernstain response over the concentration range from 9.0×10^{-7} to 1.0×10^{-2}, 1.0×10^{-6} to 1.0×10^{-2}, 1.0×10^{-6} to 1.0×10^{-2} and 5.0×10^{-6} to 1.0×10^{-2} molL^{-1} VR for electrodes I, II, III and IV, respectively, with a cationic slope of 57.56 ± 0.1, 58.45 ± 0.5, 56.17 ± 0.2 and 57.09 ± 0.7 mV decade^{-1} change in concentration for the mentioned electrodes, respectively as shown 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 ion-pair complex. The response time of the electrode(s) was tested for 1.0×10^{-7} to 1.0×10^{-1} molL^{-1} VR solutions. The electrode(s) exhibits a fast dynamic response of 25, 30, 30 and 35 s for a period of 28, 28, 30 and 25 days for electrode I, II III and IV, respectively, without significant change in the electrode(s) parameters.

Effect of soaking time and regeneration of the electrode

The performance characteristics of VR electrode(s) was studied as a function of soaking time. For this purpose, the electrode(s) was soaked in 1.0×10^{-3} molL^{-1} solution of VR and the calibration graphs were plotted after optimum soaking times 24 h for electrodes I and II, 6 h for electrode III and IV, respectively. The slope of the calibration curve was 57.56 ± 0.16, 58.45 ± 0.54, 56.17 ± 0.28 and 57.09 ± 0.74 mV decade^{-1}, at 25°C for electrodes I, II, III and IV, respectively. The electrode(s) was continuously soaked in $1x10^{-3}$ molL^{-1} solution of VR for 7, 12, 20, 25 and 35 days. The calibration plot slopes

Table 2. General working characteristics of Voriconazole sensors.

Parameter[a]	VR-PTA	VR-PMA	VR-TPB	VR-STA
Slope (mV decade^{-1})	57.56 ± 0.1	58.45 ± 0.5	56.17 ± 0.2	57.09 ± 0.7
Intercept (mV)	385.47	408.52	409.64	405.53
Correlation coefficient, *r*	0.9999	0.9998	0.9998	0.9999
Concentration range (mol L^{-1})	9.0×10^{-7} to 1.0×10^{-2}	1.0×10^{-6} to 1.0×10^{-2}	1.0×10^{-6} to 1.0×10^{-2}	5.0×10^{-6} to 1×10^{-2}
LOD (mol L^{-1})	3.9×10^{-7}	5.0×10^{-7}	4.8×10^{-7}	3.2×10^{-6}
Response time (s)	10	10	20	25
Working pH range	3-8	3-8	3-8	3-8
Lifetime (day)	28	28	30	25
Accuracy	99.46	99.16	99.54	99.77
Standard deviation	0.64	0.42	0.94	0.79
Repeatability (%)	0.72	0.58	0.82	0.67
Between day variability (%)	0.85	0.69	0.94	0.73
Robustness[b]	99.26 ± 0.62	99.74 ± 0.32	99.13 ± 0.28	99.47 ± 0.64
Ruggedness[c]	99.59 ± 0.38	99.68 ± 0.61	99.26 ± 0.36	99.47 ± 0.25

[a]Mean of six measurements; [b]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).

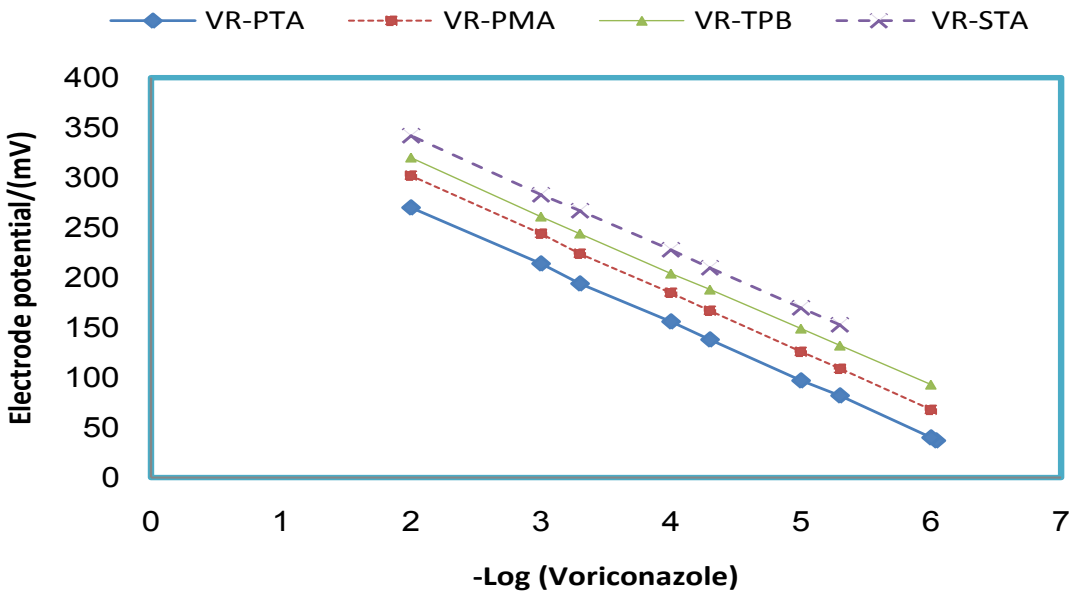

Figure 2. Typical calibration graphs of Voriconazole sensors.

decreased slightly to 55.42, 56.12, 54.52 and 53.89 mV decade^{-1} after 20 days for the mentioned four electrodes, respectively, and continued to decrease reaching 53.64, 51.74, 48.23 and 51.60 mV decade^{-1} after 35 days. This revealed 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 appeared 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 (Maha et al., 2010). The regeneration of the VR membrane was successfully achieved by soaking the exhausted electrode(s) for 24 h in a solution that was 1.0x10^{-2} molL^{-1} of each ion-pair, followed by soaking for 3 h in 1.0×10^{-2} molL^{-1} VR solution. Figures 3 and 4 show the calibration graphs for an exhausted electrode(s) (slopes 53.70 and 51.74 mV decade^{-1}) for plastic membrane electrodes I and II, respectively, and for the same electrodes after regeneration (slopes 54.40 and 53.49 mV decade^{-1}). It was found that the lifespan of the regenerated electrodes is limited to 8 h due to the ease of leaching of the lipophilic salts from the gel layer at the

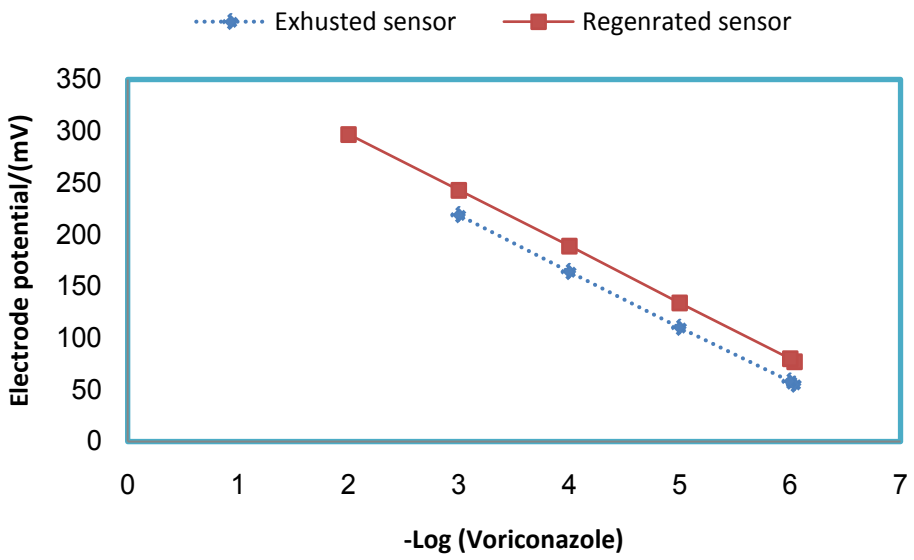

Figure 3. Regeneration of VR-PTA plastic membrane sensor.

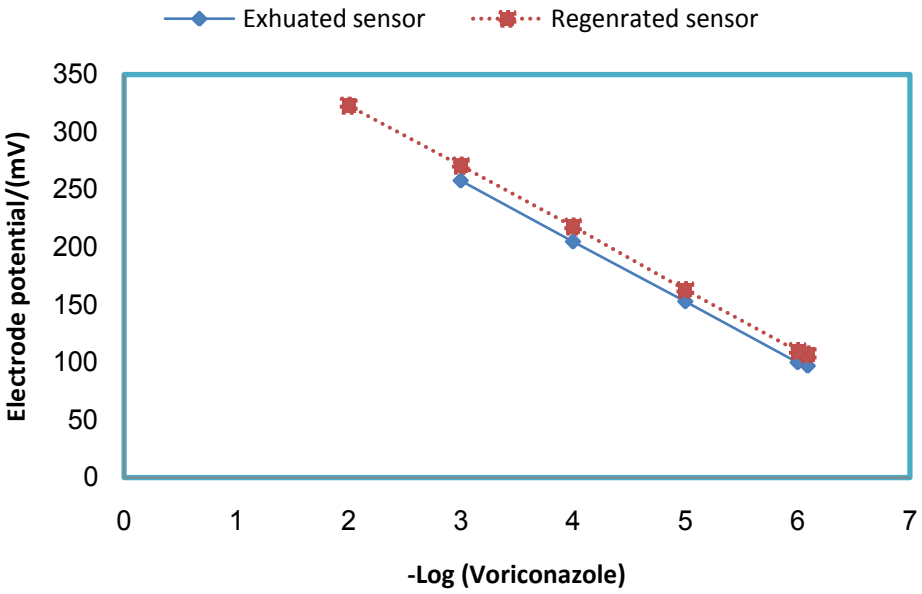

Figure 4. Regeneration of VR-PMA plastic membrane sensor.

electrode surface compared with those that are attached homogeneously to the PVC network through the solvent mediator.

Effect of pH

The effect of pH of the VR solution ($1.0x10^{-3}$ $molL^{-1}$) on the electrode(s) potential was investigated. The solution was acidified by the addition of very small volumes of 0.1 $molL^{-1}$ hydrochloric acid then the pH value was increased gradually using 0.1 $molL^{-1}$ sodium hydroxide. For each pH value, the potential was recorded and then the potential-pH curves for VR were constructed as shown in Figure 5. It was found that within the pH range of 3 to 8, the electrode(s) potential is practically independent of pH, and in this range, the electrode can be safely used for VR 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

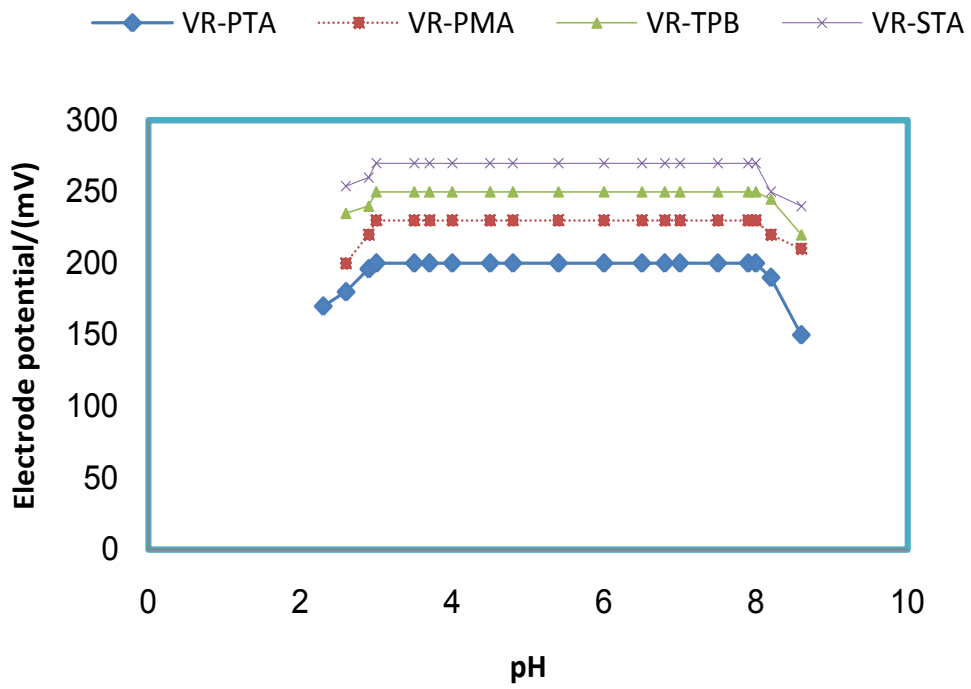

Figure 5. Effect of pH on electrode potential of Voriconazole sensors.

at pH more than 8, the response of the electrode decreased which may be attributed to increase of OH^- concentration (Nour et al., 2000).

Selectivity of the electrode

The influence of some inorganic cations, sugars and amino acids on VR electrodes was investigated. The results obtained (Table 3) reflect a very high selectivity of the investigated electrodes for the VR cation. The mechanism of selectivity is mainly based on the streospecificity 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+} do 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 some pharmacologically related compounds.

Quantification of Voriconazole

Direct potentiometric determination of VR using VR electrode(s) type I, II III and IV was performed and calculated from the calibration curves. The direct potentiometric determination of VR in pure form using the proposed electrodes gave average recovery percentage of 99.62 ± 0.61, 99.10 ± 0.82, 99.35 ± 0.79 and 99.32 ± 0.62 for electrodes I, II, III and IV, respectively. Furthermore, the results obtained were encouraging, so the proposed method was applied for the determination of VR in its pharmaceutical preparations, the results compared with the reported method (Ahmed et al., 2010), (HPLC determination of VR using mobile phase acetonitrile 0.05 $molL^{-1}$: disodium hydrogen phosphate buffer, pH 5.5 (1:1 v/v) at flow rate 1.0 mL min^{-1} using detection wavelength of 255 nm), and the results are shown in Table 4. The proposed method was successfully applied for the determination of VR in biological fluids. The results are shown in Table 5.

Validation of the proposed method

The linearity under the optimal experimental conditions was obtained between the electrode potential/mV and the logarithm of corresponding concentration of the investigated drug. The regression data, correlation coefficients (r) and other statistical parameter are shown in Table 2.

The detection limit of the investigated drug was calculated according to International Union of Pure and Applied Chemistry (IUPAC) (1976) recommendation, which stated that the detection limit is the concentration at which the measured potential differs from that

Table 3. Selectivity coefficients and tolerance values for the VR-responsive sensors.

Interferent	VR-PTA		VR-PMA		VR-TPB		VR-STA	
	SSM*	MSM**	SSM	MSM	SSM	MSM	SSM	MSM
Na^+	2.04	2.43	2.85	3.13	2.05	3.15	2.65	3.33
K^+	2.63	2.89	3.12	3.28	2.85	3.11	2.89	3.12
NH_4^+	3.40	3.97	3.41	3.64	2.75	3.28	3.25	3.56
Ca^{2+}	2.88	3.91	2.84	3.18	2.68	3.42	3.12	3.85
Mg^{2+}	3.45	3.62	3.50	3.60	2.79	3.53	3.45	3.99
Cd^{2+}	3.47	3.68	3.62	-	3.12	3.62	4.15	-
Fe^{3+}	3.89	-	3.92	-	3.30	3.58	4.36	-
Al^{3+}	4.00	-	4.11	-	3.67	-	4.28	-
Urea	4.20	-	4.28	-	4.12	-	5.01	-
Quinidine	3.01	3.12	3.10	3.34	4.20	-	4.65	-
Caffeine	3.32	3.54	3.33	3.52	3.32	3.49	3.99	-
Glucose	3.74	-	3.64	-	3.54	-	4.25	-
Lactose	4.24	-	4.12	-	3.38	-	4.36	-
Sucrose	4.57	-	4.48	-	4.68	-	4.89	-
Maltose	4.03	-	4.70	-	4.95	-	4.75	-
l-leucine	4.48	-	4.28	-	4.42	-	4.44	-
L-valin	5.31	-	4.41	-	4.58	-	3.36	3.49
l-glutamine	3.57	-	4.62	-	4.64	-	4.58	-
L-cystine	3.88	-	4.25	-	4.68	-	5.12	-
Glycin	4.14	-	4.72	-	5.74	-	4.87	-
Histamine	4.72	-	4.75	-	4.01	-	4.48	-
Histadine	5.21	-	4.52	-	4.00	-	4.25	-
Starch	4.58	-	4.00	-	4.31	-	5.22	-
Clotrimazole	5.18	-	3.91	-	4.10	-	4.18	-
Ketoconazole	4.42	-	5.12	-	5.00	-	4.65	-
Miconazole	4.17	-	4.72	-	4.82	-	4.78	-

*SSM, Separate solution method; **MSM, mixed solution method.

predicted by the linear regression by more than 18 mV (IUPAC, 1976). The values are shown in Table 2 which indicate that the proposed ion selective electrode (ISE) method is sensitive for detection of very small concentrations of VR (3.9×10^{-7}, 5.0×10^{-7}, $4.8\times10^{-7}$and 3.2×10^{-6} $molL^{-1}$).

The robustness of the proposed method was tested by investigating the capacity of the method to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage (Miller et al., 1993). The robustness of the proposed method was carried out by using phosphate buffer pH 6±1 and the percentage recoveries were 99.26 ± .62, 99.74 ± 0.32, 99.13 ± 0.28 and 99.47 ± 0.64 for the four prepared electrodes. These results were closely in agreement with those obtained from standard drug solutions (Table 2). While the ruggedness of the proposed method was investigated by measuring the degree of reproducibility at test results obtained by the analysis of the same samples under a variety of conditions such as different laboratories, analysts and instruments. The reproducibility upon using another model of pH-meter (Jenway 3510) was indicated by the results obtained (Table 2).

The accuracy of the proposed method was investigated by the determination of VR in spiked placebo samples prepared from serial concentrations of VR reference standards. The results shown in Table 6 show that the proposed method is an accurate one for the determination of VR in its pharmaceutical preparations without interfering from the coformulated adjuvants as indicated by the percentage recoveries values of 99.46 ± 0.63, 99.16 ± 0.42, 99.54 ± 0.93 and 99.77 ± 0.78.

The precision of the proposed ISE method, measured as RSD% was tested by repeating the proposed method for determination of the investigated drug in its pharmaceutical preparations to nine replicates. The RSD% values for the repeated determinations were 0.352, 0.458, 0.864 and 0.414% for determination of VR in Vfend® 50 mg/tablet using the four mentioned electrodes, respectively. The RSD% values are less than 2% indicating good precision.

Table 4. Statistical treatment of the data obtained for the determination of Voriconazole by the proposed and reported methods.

Pharmaceutical preparations	Reported method[a]	VR-PTA		VR-PMA		VR-TPB		VR-STA	
		Calibration method	Standard addition method	Calibration method	Standard addition method	Calibration method	Standard addition method	Calibration method	Standard addition method
Pure solution									
Mean	99.38	99.62		99.10		99.35		99.32	
SD	0.92	0.61		0.82		0.79		0.62	
SE	0.37	0.25	-	0.33	-	0.30	-	0.23	-
t-test		0.53(2.23)*		0.56(2.23)*		0.06(2.20)*		0.14(2.20)*	
F-test		2.29(5.05)*		1.26(5.05)*		1.37(4.39)*		2.23(4.39)*	
Vfend®-50 mg/tablet									
Mean	99.56	99.13	99.44	99.62	99.38	99.12	99.46	99.26	99.18
SD	0.39	0.57	0.62	0.48	0.56	0.47	0.52	0.68	0.46
SE[b]	0.16	0.23	0.22	0.19	0.22	0.19	0.10	0.28	0.19
t-test		1.53 (2.23)[c]	0.44 (2.23)[c]	0.24 (2.23)[c]	0.66 (2.23)[c]	1.77 (2.23)[c]	0.53 (2.23)[c]	0.93 (2.23)[c]	1.53 (2.23)[c]
F-test		2.13 (5.05)[c]	2.56 (5.05)[c]	1.54 (5.05)[c]	2.07 (5.05)[c]	1.47 (5.05)[c]	1.80 (5.05)[c]	3.08 (5.05)[c]	1.41 (5.05)[c]

[a](Ahmed et al., 2010), [b]SE=%error= %RSD/√n, [c]The figures in parentheses are the tabulated t- and F- test at P = 0.05 (Miller et al., 1993).

Electrode response in pharmaceuticals and biological fluids

This work proposed a fast, simple, easy, sensitive and straightforward potentiometric method to determine VR in dosage forms without the need of prior separation and preconcentration or derivatization procedures. The potential of the VR sensors showed no significant difference of response time between aqueous solution of pure drug and its solutions from pharmaceutical preparations and biological fluids. The proposed method described good accuracy and precision for the quality control tests. The content uniformity assay showed that the RSD<1%, with mean standard deviation 99.22 ± 0.24, 99.18 ± 0.65, 99.36 ± 48 and 99.33 ± 0.28 for electrodes I, II, III and IV, respectively.

Conclusion

The described potentiometric method has simple workup procedure and requires no sophisticated instrumentation. It determines only the therapeutically active undegraded drug in the presence of its excipients without separation. The results obtained also show that the constructed sensors provide response suitable for analytical use in the determination of VR 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. It offers distinct advantages in rapidity and simplicity. It is suitable for routine determination of VR in quality control laboratories for content uniformity assay of tablets. This conclusion is justified by the results obtained from the analysis of pharmaceutical preparations and biological fluids for which precise and accurate recoveries were obtained.

ACKNOWLEDGEMENT

This project was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center.

Table 5. Determination of VR spiked in human serum and urine using VR sensors

Statistical parameter	VR-PTA	VR-PMA	VR-TPB	VR-STA
Urine sample				
Mean	99.02	99.19	98.99	99.13
n	6	7	6	6
SD	0.53	0.40	0.85	0.60
%RSD	0.53	0.41	0.86	0.61
Serum sample				
Mean	99.10	99.19	98.72	98.58
n	5	5	5	5
SD	0.97	0.67	0.39	0.42
%RSD	0.98	0.78	0.40	0.43

Table 6. Determination of VR in spiked placebo samples using VR sensors.

Statistical parameter	VR-PTA	VR-PMA	VR-TPB	VR-STA
Mean	99.46	99.16	99.54	99.77
n	9	9	9	9
SD	0.63	0.42	0.93	0.78
%RSD	0.64	0.42	0.94	0.79

REFERENCES

Adams AIH, Steppe M, Froehlich PE, Bergold AM (2006).Comparison of microbiological and UV-spectrophotometric assays for determination of voriconazole in tablets. J. AOAC. Int., 89: 960-965.

Adel FS, Yousry MI, Rasha ME (2001). Repretrol plastic membrane ion selective electrodes based on its individual and mixed ion exchangers with phosphotungstic and /or phosphomolybdic acids. Michrochem., 69: 189-197.

Ahmed BE, Abdalla AS, Magda YE (2010). Development and validation of a HPLC method for the determination of voriconazole and its degradation products in pharmaceutical formulation. Acta. Pharm. Sci., 52: 229-238.

Araujo BV, Conrado DJ, Palma EC, Dalla CT (2007). Validation of rapid and simple LC- MS/MS method for determination of voriconazole in rat plasma. J. Pharm. Biomed. Anal., 44: 985-990.

Buck RP, Linder E (1994). Recommendation for nomenclature of ion-selective electrodes. Pure Appl. Chem., 66: 2527-2536.

Graggs A, Moody GJ, Thomas JDR (1974). PVC matrix membrane ion-selective electrodes. Construction and laboratory experiments. J. Chem. Ed., 5: 541-544.

Gu P, Li Y (2009). Development and validation of stability-indicating HPLC method for determination of voriconazole and its related substances. J. Chromatogr. Sci., 47: 594-598.

International Union of Pure and Applied Chemistry (IUPAC) - Information Bulletin (1976). Recommendations for nomenclature of ion-selective electrodes, 48: 127-132.

Kahke K, Langmann P, Schirmer D, Lenker U, Keller D, Helle A, Klinker H, Heinz W (2009). Simultaneous determination of voriconazole and pasaconazole concentrations in human plasma by high-performance liquid chromatography. J. Antimicr. Agents Chemother., 53: 3140-3142.

Khoschsorur G, Fruehwirth F, Zelzer S (2005). Isocratic high-performance liquid chromatographic method with ultraviolet detection for simultaneous determination of levels of voriconazole and itraconazole and its hydroxyl metabolite in human serum. Antimicr. Agents Chemoth., 49: 3569-3571.

Ma TS, Hassan SSM (1982).Organic Analysis Using Ion Selective Electrode. Vols. I and II, Academic Press, London, UK.

Maha ET, Sawsan R, Magda EM, Shalaby AA (2010). Construction and Optimization of Selective Membrane Electrodes for Determination of Doxepin Hydrochloride in Pharmaceutical Preparations and Biological Fluids. Korean J. Chem., 54: 1-10.

Michael C, Teichert J, Preiss R (2008). Determination of voriconazole in human plasma and saliva using high-performance liquid chromatography with fluorescence detection. J. Chromatogr., B, 865: 74-80.

Miller JC, JN, Miller JN (1993). Statistics for Analytical Chemistry, third ed., Ellis Horwood-Prentice Hall, Chichester. pp. 543-544.

Moody G, Thomas JDR (1989). Ion selective electrodes from glasses to crystals and crowns. Am. Chem. Sci., 390: 303-317.

Nour T, Abdel G, Mahmoud SR, Rasha ME (2000). Salbutamol plastic membrane electrodes based on individual and mixed ion-exchangers of salbutamolium phosphotungstate and phosphomolybdate. Analyst, 125: 1129-1133.

Oesh U, Simon W (1980). Lifetime of neutral carrier based ion-selective liquid. Membr. Electr. Anal. Chem., 52: 692-700.

Pehourcq F, Jarry C, Bannwarth B (2004). Direct HPLC micro method for the determination of voriconazole in plasma using internal surface reversed-phase column. Biomed. Chromatogr., 18: 719-722.

Product information (Pfizer, Inc.) (2002). Vfend® (voriconazole). Pfizer, Inc. New York, NY.

Shoukry AF, Issa YM, El-Nashar RM (2001).Reproterol plastic membrane ion-exchangers with phosphotungstic and /or phosphomolybdic acids. J. Microchim., 69: 189-197.

Sinubabu G, Raju CAI, Sarath N, Kumar PK, Rao JVLN, Seshagiri (2007). Development and validation of a HPLC method for the determination of voriconazole in pharmaceutical formulation using an experimental design. Talanta, 71: 1424-1429.

Theurillat R, Zimmerli S, Thormann W (2010). Determination of voriconazole in human serum and plasma by micellarelectrokinetic chromatography. J. Pharm. Biomed. Anal., 53: 1313-1318.

Xiong X, Duan J, Zhai S, Wang L, Lan X (2010). Fast and reliable determination of voriconazole in human plasma by LC-APCI-MS/MS. Biosci. Biotechnol. Biochem., 74: 2151-2153.