Research Article

Determination of Venlafaxine Hydrochloride in Biological Fluids Using Prepared Ion Selective Electrode

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Accepted on: 10-03-2016; **Finalized on:** 31-03-2016.

ABSTRACT

A coated-wire ion selective electrode based on ion-pair complex of venlafaxine (VEN) with phosphomolybdic acid (PMA) as electroactive material in the presence of dioctylphthalate (DOP) as the plasticizing solvent mediator was prepared. The electrode showed a fast, stable and Nernstian response over a wide venlafaxine concentration range (1.0×10^{-5} to 1.0×10^{-2} mol L^{-1}) with a slope of 58.70 mV dec^{-1} of concentration. The influences of membrane composition, temperature, pH of the test solution, and foreign ions on the electrode performance were investigated. The electrode was suitable for use in aqueous solutions in a wide pH range of 4.0 to 8.0. The standard electrode potentials, $E°$, were determined at different temperatures and used to calculate the isothermal temperature coefficient ($dE°/dT$) of the electrode. The proposed sensor displayed useful analytical characteristics for the determination of VEN in pure solutions and in biological fluids such as plasma and urine samples using the standard additions method.

Keywords: Venlafaxine hydrochloride (VEN); Biological fluids; Potentiometry.

INTRODUCTION

Venlafaxine hydrochloride, (1-[2-dimethylamino)-1-(4-methoxy phenyl) ethyl] cyclohexanol) hydrochloride (*Figure 1*) is a third generation antidepressant[1]. The drug is a potent inhibitor of neuronal serotonin and norepinephrine reuptake, but a weak inhibitor of dopamine reuptake. Venlafaxine is well absorbed, with peak plasma concentrations occurring approximately 2 hours after dosing. It is extensively metabolised, to Odesmethylvenlafaxine, the only major active metabolite[2]. The reported methods for venlafaxine analyses in biological fluids included GC [3,4], HPLC [5–9], UV detection[10,11] and capillary electrophoresis[12]. Although, those methods are very sensitive, they are not adapted for in situ and real time detection of DL as they are time consuming, involve expensive apparatus and require skilled technicians.

Potentiometric methods based on this technique are simple, rapid, low detection limit, good accuracy, wide concentration range, applicability to colored and turbid solutions and low cost without separation or pretreatment procedures[13]. The conventional Polyvinylchloride (PVC) membrane ion-selective electrodes have found a wide range of applications in biological fluids; however they still have certain inherent limitations. Drawbacks in the use of PVC electrodes were arisen from the time consuming and inconsistent manual fabrication typically employed as well as short lifetime of these electrodes[14]. To overcome the aforementioned difficulties in PVC membrane electrodes, new kinds of all solid-state ones (without internal reference solution) allowing electrode miniaturization capability were introduced. Coated wire electrodes (CWEs) and coated graphite electrodes are examples of these sensors design; however the potential drift and the poor adhesion of the membrane to the metal substrate are main drawbacks of these electrodes[15].

This work describes construction and investigation of performance characteristics of ISE based on coated wire electrode for the determination of venlafaxine hydrochloride in pure solutions and in biological fluids such as plasma and urine samples using the standard additions method.

Figure 1: Chemical structure of venlafaxine hydrochloride.

Experimental

Materials and Equipment

All chemicals were of analytical grade, and double distilled water was used throughout the experiments. Venlafaxine hydrochloride was obtained from Sigma Co. phosphomolybdic acid (PMA), poly (vinyl chloride) of high molecular weight (PVC), tetrahydrofuran (THF) and dioctylphthalate (DOP) were purchased from Merck (Germany). Stock venlafaxine hydrochloride solution (1.0×10^{-2} mol L^{-1}) was prepared daily by dissolving an appropriate amount of the drug in double distilled water.

More dilute solutions were prepared by appropriate dilution.

To investigate the selectivity of the proposed electrode towards inorganic cations, amino acids and sugars, 1.0×10^{-2} mol L^{-1} solution of each of the following ions were prepared: Na^{+}, Co^{2+} and Cr^{3+}. Also 1.0×10^{-2} mol L^{-1} solution of lactose, glucose, valine and, glycine were prepared.

Potentiometric measurements were carried out with an Orion (Cambridge, MA, USA) Model 701 A digital pH/mV-meter. The electrochemical system may be represented as follows:

Copper (wire)-PVC membrane | sample solution || Ag-AgCl, KC1 (satd.)

Preparation of the ion-pair

The ion-pair compound, VEN- PMA was prepared by slow addition of 50 mL of 1.0×10^{-2} mol L^{-1} phosphomolybdic acid solution to 50 mL of 1.0×10^{-2} mol L^{-1} venlafaxine HCl under stirring for 15 min. The resulting precipitate was filtered, washed with cold distilled water several times and dried in room temperature for 2 days. The chemical composition of associate complex has a molar ratio of 3:1 for VEN:PMA and was ascertained by elemental analysis.

Construction of electrode

To make a wire coated electrode the copper wire of 2.0 mm diameter and 12 cm length were tightly insulated by polyethylene tube leaving 1.0 cm at one end of the coating and 0.5 cm at the other end for connection. The coating solutions were prepared by dissolving varying amounts of Polyvinylchloride (PVC), dioctylphthalate, DOP (plasticizer), and the VEN–PMA in the least amount of tetrahydrofuran possible (4 ml). Prior to coating, the polished copper surface was washed with a detergent and water, thoroughly rinsed with de-ionized water, and dried with acetone. Afterwards, the copper wire was coated by quickly dipping it into the coating solutions several times and allowing the film left on the wire to dry for about 5 min. The process was repeated several times until a plastic membrane of approximately 1.0 mm thickness was formed. Then, it was soaked in a 1.0×10^{-3} mol L^{-1} of venlafaxine HCl solution for 3 h. When not in use, the electrode was stored in air.

Construction of the Calibration Graphs

Suitable increments of standard VEN solution were added to 50.0 ml doubly distilled water so as to cover the concentration range from 1.0×10^{-6} - 1.0×10^{-2} mol L^{-1}. In this solution the sensor and reference electrode were immersed and the *e.m.f*. values were recorded at 25 °C after each addition. The electrode was washed with double distilled water and dried between measurements. The electrode potentials, E_{elec}, were calculated from the *e.m.f.* values and plotted versus negative logarithmic concentration of VEN, Slopes of the resulting calibration curves were calculated.

The process was repeated at 30, 35, 40, 45 and 50 °C. No significant change in the performance of the electrode was observed during this period, but after two months, a gradual decrease occurred in the slope.

Selectivity of the electrode

Selectivity coefficients were determined by the separate solution method, in which the following equation was applied.

$$Log\, K^{pot.}_{VEN,B^{z+}} = \left(E_2 - E_1\right)/S + \log[VEN] - \log\left[B^{z+}\right]^{1/z^{+}}$$

eq. (1)

Where E_1 and E_2 are the electrode potentials of solutions of VEN and interfering cation, B^{z+}, respectively (both of the same concentration, 1.0×10^{-2} M) and S is the slope of the calibration graph[16].

Standard Addition Method

The standard addition method[17] was applied, in which small increments of a standard VEN solution 1×10^{-2} M were added to 50 ml aliquot samples of various drug concentrations. The change in potential reading at a constant temperature of 25˚C was recorded for each increment and used to calculate the concentration of the drug sample solution using the following equation:

$$C_x = C_s\left(\frac{V_s}{V_s+V_s}\right)\left(10^{n\left(\Delta E/S\right)} - \frac{V_x}{V_x+V_s}\right)^{-1}$$

eq. (2)

where Cx and Vx are the concentration and volume of the unknown, respectively, Cs and Vs are the concentration and volume of the standard, respectively, S is the slope of the calibration graph, and ΔE is the change in potential due to the addition of the standards.

Determination of VEN in biological fluids

Different amounts of venlafaxine hydrochloride, and 5 mL of plasma or urine of a healthy person were transferred to 50 mL measuring flask and completed to the mark using doubly distilled water. The contents of the measuring flask were transferred to a 100 mL beaker, and subjected to potentiometric determination of venlafaxine hydrochloride by standard addition method.

RESULTS AND DISCUSSION

Influence of membrane composition

Five coating membrane compositions were investigated as given in **Table 1**. The best performance was exhibited by membrane *C** with ion pair, DOP and PVC in the ratio 7:46.5:46.5, respectively. This membrane showed a nearly Nernestian response with slope of 58.70 mV/decade and a linear concentration range 1.0×10^{-5}–1.0×10^{-2} mol/L (Figure 2). Membranes *D* and *E* showed a sub-Nernestian response with slope of 55.80 and 53.20 mV/decade, respectively.

However, they still have sufficient sensitivity with good linear range and can thus be used for the determination of venlafaxine in solution. Since membrane *C** has the highest sensitivity among all studied compositions, it was studied in detail as VEN selective electrode and all further investigations were carried out with this particular membrane.

Table 1: Composition of the different VEN-PMA representative membranes and slopes of the corresponding calibration graphs at 25 °C.

Membrane	Composition % (w/w)			Slope mV/decade	Linear range	RSD[a] (%)
	Ion Pair	PVC	DOP			
A	2.0	49.0	49.0	30.87	4.3×10^{-4}- 1.0×10^{-2}	0.67
B	4.0	48.0	48.0	43.84	7.0×10^{-4}- 1.0×10^{-2}	0.87
C*	7.0	46.5	46.5	58.70	1.0×10^{-5}- 1.0×10^{-2}	1.18
D	9.0	45.5	45.5	55.80	5.0×10^{-5}- 3.0×10^{-2}	0.98
E	11.0	44.5	44.5	53.20	4.0×10^{-5}- 1.0×10^{-2}	0.63

[a]Relative standard deviation (three determinations)

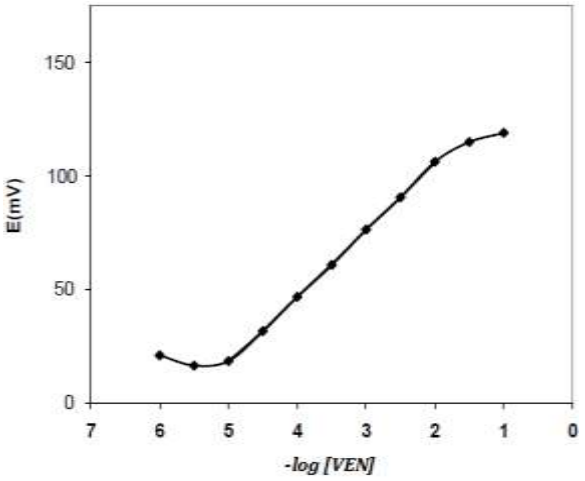

Figure 2: Typical calibration curve of CWE electrode

Response Time

The response time is the time which elapses between the instant when an ion-selective electrode and a reference electrode (ISE_{cell}) are brought into contact with a sample solution. The response time was tested for 1.0×10^{-6} - 1.0×10^{-2} mol L^{-1} VEN solutions. This electrode exhibits a fast dynamic response of about 15-20 s over the whole concentration range of VEN drug. The electrode was used for about two months. During these times, the detection limit and the slope of the electrode remained almost constant. Subsequently, the electrochemical behavior of the electrode gradually deteriorated. This would be due to aging effect and leaching of the ion pair and the solvent mediator from the membrane into the solution by time.

pH Effect on the electrode response

Since pK_a of venlafaxine is 9.4, therefore at pH 8.4 venlafaxine is nearly completely ionized, i.e. venlafaxine will be in the cationic form. The influence of pH on the response of the CWE was examined for the 1.0×10^{-3} and 1.0×10^{-4} mol L^{-1} venlafaxine solutions. The pH was adjusted by adding small volumes of (0.1–1.0) mol L^{-1} HCl or NaOH to the test solutions and the variation in potential was followed.

The results showed that the potential remained constant despite the pH change in the range of 4.0 to 8.0, which indicates the applicability of this electrode in the specified pH range. Relatively noteworthy fluctuations in the potential *vs.* pH behavior took place below and above the formerly stated pH limits. In detail, the fluctuations above the pH value of 8.0 might be justified by removing the positive charge on the drug molecule. Fluctuations below the pH value of 4.0 were caused by removal of the ion-pair in the membrane or analyte in the solution.

Selectivity of the electrode

The selectivity coefficients $K^{pot}_{VEN^+,B}$ were determined by the separate solution method[17]. The influence of a variety of some inorganic cations, sugars and amino acids on the VEN electrode was investigated. The results reflect a very high selectivity of the investigated electrode for the venlafaxine cation. The smaller the value of $K^{pot}_{VEN^+,B}$, the better the sensitivity of the electrode in the presence of the interfering ions. In case of a good ion-selective electrode, it should have a value of about 10^{-4}. Table 2 summarizes the selectivity coefficient factor of the liquid membrane electrode for some common cations, sugars and amino acids that are expected to be present in the dosage forms of the drug.

The selectivity mechanism is mainly based on the sterospecificity and electrostatic environment, and is dependent on how much fitting is present between the

locations of the lipophilicity sites in the two competing species on the bathing solution side and those present in the receptor of the ion-exchanger[18]. In other words, the distribution of the active components situated at the electrode surface allow the primary ions to react but do not enter the membrane phase under zero-current conditions and this explained the fact that the electrode surface is not completely flat but has depressions and hills[19].

The inorganic cations did not interfere due to the differences in their ionic size, mobility and permeability. Also, the smaller the energy of hydration of the cation facilitated a greater response of the membrane. In the case of sugar and amino acid, the high selectivity is mainly attributed to the difference in polarity and lipophilic nature of their molecules relative to venlafaxine hydrochloride.

Table 2: Selectivity coefficient values of the CWE electrode.

Interfering ions (B)	Selectivity Coefficient $K^{pot}_{VEN^+,B}$
Na^+	-
Co^{2+}	-
Cr^{2+}	-
Glucose	1.25×10^{-3}
Lactose	3.32×10^{-4}
Glycine	2.18×10^{-3}
Valine	1.37×10^{-3}

Effect of Temperature of the test solution

To study the thermal stability of the electrode, the electrode potential of 10^{-5}–10^{-2} M VEN drug solutions were determined in 25, 30, 35, 40, 45 and 50 °C and the calibration graphs (cell potential, E_{cell} *vs* *pVEN*) were constructed. The standard electrode potentials ($E°$) were determined, as the intercepts of the calibration graphs at pVEN = 0, and used to obtain the isothermal temperature coefficient ($dE°/dT$) of the electrode by aid of the following equation[20].

$$E° = E°_{25.0} + (dE°/dT)\,(t - 25.0) \qquad \textbf{eq. (3)}$$

A plot of E^o *vs* $(t - 25.0)$ gave a straight line, the slope of which was taken as the isothermal temperature coefficient.

It amounts to 0.00060 V per °C, revealing a fairly good thermal stability of the electrode within the investigated temperature range and show no deviation from the theoretical Nernstian behavior.

Analytical applications

To assess the applicability of the proposed electrode, venlafaxine hydrochloride was determined in pure solution, urine and plasma samples, by applying the standard addition method.

The obtained average recovery and relative standard deviation values are summarized in Tables 4 and 5, which reflect the high percentage recovery without pretreatment of the samples.

Table 4: Test of precision of the standard addition and potentiometric titration methods on pure venlafaxine hydrochloride.

Amount taken (µg\ml)	Recovery %	RSD*
3.0	99.12	1.15
9.0	98.76	0.87
75.0	97.78	1.73
225.0	99.90	0.98
Mean	98.89	1.18
Mean ± SD	98.89±1.18	

Table 5: Standard additions method for determination of VEN in urine and plasma samples.

Sample	Urine	Plasma
Amount taken (µg\ml)	9.0	9.0
	75.0	75.0
	225.0	225.0
Recovery %	97.9	98.9
	99.8	102.12
	100.1	99.3
RSD*	0.79	1.34
	0.83	0.67
	1.54	0.98
Mean ± SD	99.27±1.05	101.11±1.00

* RSD (three determinations)

CONCLUSION

The prepared CWE ion-selective electrode incorporating VEN-PMA as a sensing material and DOP as solvent mediator could be used to determine VEN drug in the concentration range 1.0×10^{-2} - 1.0×10^{-5} mol L^{-1} with a slope of 58.70 mV/decade. This electrode is very easy to prepare, show high selectivity and sensitivity, wide dynamic range. The working pH range of this electrode is 4.0–8.0. The electrode showed a very good selectivity to VEN in the presence of various common inorganic cations, sugars and amino acids. The response time for the static potential was found to be significantly low, hence the electrode can be also applied for the determination of VEN in pure solutions and in biological fluids.

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Source of Support: Nil, **Conflict of Interest:** None.