Anal. Bioanal. Electrochem., Vol. 10, No. 11, 2018, 1414-1425

Analytical & Bioanalytical Electrochemistry

2018 by CEE
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Full Paper

Functionalized β-cyclodextrin-based Potentiometric Membrane for the Selective Determination of Vildagliptin in Presence of its In-process Impurity

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Received: 16 November 2018 / Received in revised form: 20 September 2018 / Accepted: 9 October 2018 / Published online: 30 November 2018

Abstract- A novel vildagliptin selective electrode has been investigated using tetrakis (p-chlorophenyl) borate as cation exchanger in polymeric matrix of polyvinyl chloride plasticized with orth-nitro phenyl octyl ether. The proposed sensor was fabricated using hydroxy propyl β-cyclodextrin as ionophore and compared to ionophore free sensor. A stable and reliable response of vildagliptin within the concentration range of 1×10^{-1}to1×10^{-4} mol.L^{-1} was obtained on using ionophore based sensor with detection limit down to 5×10-6 mol.L^{-1}. Nernstian slope was 55.3 mV/decade over in aqueous medium (pH 7) was observed with fast response time. The selectivity coefficients calculated for L-proline as in-process impurity and different inorganic cations indicated excellent selectivity for vildagliptin. Hydroxy propyl β-cyclodextrin sensor displayed useful analytical characteristics for the determination of vildagliptin. The fabricated ionophore based sensor was validated and found to be accurate and precise compared with the manufacturer's gradient HPLC method. Moreover, this potentiometric has advantage over previously published methods where no sample pretreatment is required.

Keywords– Potentiometer, Ionophore, Impurity, Vildagliptin

1. INTRODUCTION

Selective determination of active pharmaceutical ingredients in presence of impurities in bulk drugs and pharmaceutical formulations are important regulatory requirements for new drugs [1]. Impurities may originate from several sources can be attributed to presence of impurities, for instance, degradation products, excipients and incomplete reactions during the synthesis [2]. Precursors are probably not depleted in the last case and hence a trace amount can be detected as impurities in the final pure form of active ingredient. Vildagliptin (VIL) is chemically designed as (S)-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyrrolidine-2-carbonitrile. Vildagliptin is well known inhibitor of dipeptidylpeptidase-4 and used as oral anti-diabetic agent for treatment of type 2 diabetes mellitus [3,4]. Novartis has lunched this hypoglycemic drug in 2000 [5]. L-proline is involved in the starting chemical reaction of vildagliptin synthesis [6]. Vildagliptin is unofficial in any pharmacopeia. Literature survey reveals that single determination of vildagliptin is obtained by HPLC in plasma and pharmaceutical formulations [7-10]. In addition, some charge transfer complexes of vildagliptin have been used for its spectrophotometric [11-13] and spectroflourimetric analysis [13]. Determination of vildagliptin with its in process impurities includes HPLC [14] and enantiomer selective determination using capillary electrophoresis [15]. Stability-indicating method of vildagliptin determination includes HPLC [16] and TLC [17]. None of the reported methods consider a simple and convenient analytical technique for selective determination of VIL without interference of its L-proline impurity.

Advancement in potentiometric sensors enables the selective determination of vast active pharmaceutical ingredients [18,19]. Ion transport across these electrodes is controlled by the membrane components for selective determination of ionic analytes. Components in membranes usually include ionic species with or without complexing agents. Phenyl borate salts, for instance, are widely used ionic species in membrane sensors owing to its apparent lipophilicity. Incorporation of such negative ionic sites renders the membrane responsive to positively charged counter ions. On the other hand, inclusion complex formed between target analyte and ionophore favor the selective transport of this analyte across the membrane and hence improve its selectivity [20]. Cyclodextrin are well known to accommodate wide variety of organic molecules to form stable host-guest inclusion complexes assemblies in their hydrophobic cavity while exhibiting high molecular selectivity [21,22]. Ion selective electrodes are relatively simple and cheap to develop analytical technique. The present work describes the use of (2-hydroxypropyl)-β-cyclodextrin as neutral ionophore for the development of novel sensor for the determination of vildagliptin. Selectivity of the sensor was evaluated in presence of L-proline as a starting material for vildagliptin synthesis.

2. EXPERIMENTAL

2.1. Materials and chemicals

Vildagliptin pure form was kindly supplied by Mash premiere for pharmaceutical industry, Cairo, Egypt, its purity was 99.17% according to the manufacturer's HPLC method using C18 column thermostated at 50℃ and ammonium di-hydrogen orthophosphate buffer pH 6.8 and acetonitrile was used as a mobile phase with gradient elution at flow rate 1 $ml.min^{-1}$ and UV detection at 210 nm. Pharmaceutical formulation Galvus® tablets lot no.B5293 (Novartis Company) was labeled to contain 50 mg per tablet and purchased from local commercial market. All chemicals and reagents used were of analytical grade and water for injection (Otsuka). Potasium tetrakis(p-chlorophenyl)borate (TpClPB), (2-hydroxypropyl)-β-cyclodextrin (HPβCD) and orth-nitro phenyl octyl ether (NPOE) were purchased from Sigma-Aldrich (Steinheim, Germany). Poly (vinyl chloride) (PVC) was obtained from Fluka Chemie GmbH (St. Louis, MO, USA). Tetrahydrofuran (THF) was obtained from BDH (Poole, England). 0.1 $mol.L^{-1}$ HCl and 0.1 $mol.L^{-1}$ NaOH standard solutions have been used for pH adjustment.

2.2. Fabrication of membrane sensor

Ionophore-free membranes were prepared by mixing 10 mg tetrakis p-chloro tetra phenyl borate as ion exchanger in about 390 mg NPOE as a plasticizer and 190 mg PVC. Another ionophore based membranes were prepared using 10 mg (2-hydroxypropyl)-βcyclodextrin as an ionophore. Each cocktail was dissolved in THF (4 mL) and poured into a Petri dish (50 mm i.d.). The membranes were left to stand overnight for slow solvent evaporation at room temperature. Master membrane of about 0.1 mm thickness was obtained. Disks were cut (about 8.0 mm in diameter) and cemented to flat end of PVC tubing with the aid of THF. A mixed solution consisting of equal volumes of 1×10^{-4} $mol.L^{-1}$ vildagliptin and 1×10^{-4} $mol.L^{-1}$ sodium chloride was used as an internal reference solution for the sensors. Ag/AgCl electrode (3 mm diameter) was used as an internal reference electrode. The sensor was conditioned by soaking in a solution of 1×10^{-4} $mol.L^{-1}$ vildagliptin for 24 h and stored in the same solution when not in use.

2.3. Potentiometric measurements

All the measurements were carried out using a potentiometric cell comprising the proposed ion-selective electrode and Ag/AgCl double-junction-type external reference electrode [Orion 900200 (Thermo Scientific, Waltham, MA); 3 M KCl saturated with AgCl as an inner filling solution, and 10% KNO3 as a bridge electrolyte]. Potential was measured using Jenway digital ion analyzer (Model: 3505; United Kingdom) and WiseStir® (Model:

MSH-20D) made by thermo line company in USA. Adjustments of pH were carried out using a Jenway pH glass electrode.

2.4. Sensor calibration

Calibration of the conditioned sensors were performed by separately immersing them in conjunction with a double junction Ag/AgCl reference electrode in different vildagliptin standard solutions (1×10^{-4} to 1×10^{-1} mol.L^{-1}) pH 7±0.2 adjusted with 0.1 M HCl and completed to volume with water for injection. The sensor was allowed to equilibrate while stirring and recording the potential difference (Electromotive force) in readings within ±1 mV. Membrane sensor was washed between measurements with water for injection. The recorded potentials were plotted as a function of logarithm vildagliptin concentrations at 25 °C.

2.5. Effect of pH

The effect of pH on the response of (HPβCD) sensor (potential) was investigated using 1×10^{-3} mol.L^{-1} and 1×10^{-4} mol.L^{-1} aqueous vildagliptin standard solutions at different pH values, ranging from 2.0 to 11.0. The obtained potential at each pH value was recorded.

2.6. Sensor selectivity

Bakker's protocol [23] was followed by plotting potential values measured by the ionophore based sensor as a function of L-proline (10^{-2}-10^{-4} mol .L^{-1}) to determine the unbiased selectivity. The potentiometric selectivity coefficients ($K_{A,\,B\,pot}$) that is independent on the ions concentration were evaluated according to International Union of Pure and Applied Chemistry (IUPAC) guidelines using the separate solutions method [24]. The potentials were measured for vildagliptin cation (A) and interfering ion (B) standard solutions, both having the same activity ($a_A = a_B$) in their linear response. Different interfering ions with concentrations of 1×10^{-3} mol.L^{-1} in water were also used to assess the membrane selectivity.

Potentiometric measurements were carried out in different synthetic laboratory mixtures containing a fixed concentration of L-proline (1×10^{-4} mol.L^{-1}) while varying concentrations of vildagliptin in water for injection. The potential of each mixture was recorded with ionophore based sensor and the concentration of vildagliptin was calculated from the corresponding regression equation.

2.7. Application to Galvus® tablets

Twenty tablets were accurately weighed, crushed to a fine powder. A portion of Galvus® tablets powder equivalent to 76 mg of vildagliptin were accurately transferred into separate

25- mL volumetric flask, and the volume was filled to the mark with water. Ten-fold dilution of the prepared solution was applied using water, and then the potentials of the prepared aqueous solutions of tablets were measured. The concentrations of vildagliptin were calculated from the corresponding regression equation.

3. RESULT AND DISCUSSION

VIL possesses a basic secondary amino group pKa ~9 [25]. pK_a of vildagliptin was practically confirmed in our laboratory by indirect potentiometry (Fig. 1). Therefore at pH values below 9 the basic center of the drug will be positively charged and facilitates the use of potentiometric sensor for its analysis.

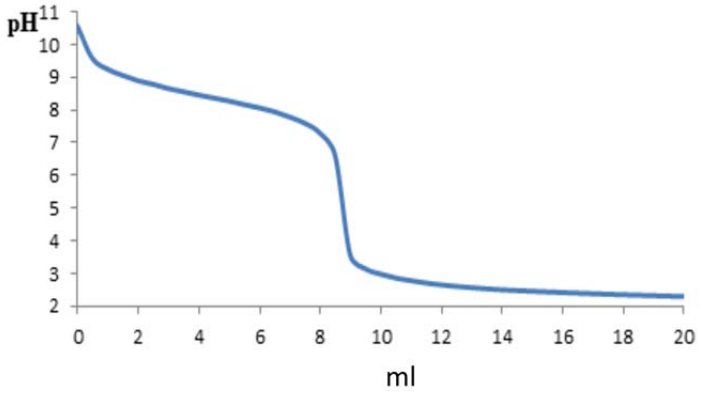

Fig. 1. Indirect potentiometric determination of vildagliptin pK_a

Anionic phenyl borate salts are suitable ion pair VIL sensor. We used chloro derivative of phenyl borate owing to its larger lipophilicity that possibly improve the electrochemical responses. On the other hand, NPOE was the best plasticizer with respect to electrochemical characteristics. This investigation is in accordance with some other reported membrane sensors for drug analyses [26-29]. Fig. 2 represents chemical structures of vildagliptin and L-proline where L-proline as in-process impurity, a zwieter ion containing both acidic (pKa~2) and basic (pKa~10) functionalities with an isoelectric point at about pH 6. Inclusion complexation is of current interest in host-guest and offers a promising approach to chemical sensing [30]. The present work evaluates the possibility of using 8-hydroxy propyl β-cyclodextrin (fig 2 c) as an ionophore in the preparation of vildagliptin selective electrode in polyvinyl chloride (ca.32%) as a polymeric matrix with NPOE (65%) was used as an optimum membrane composition.

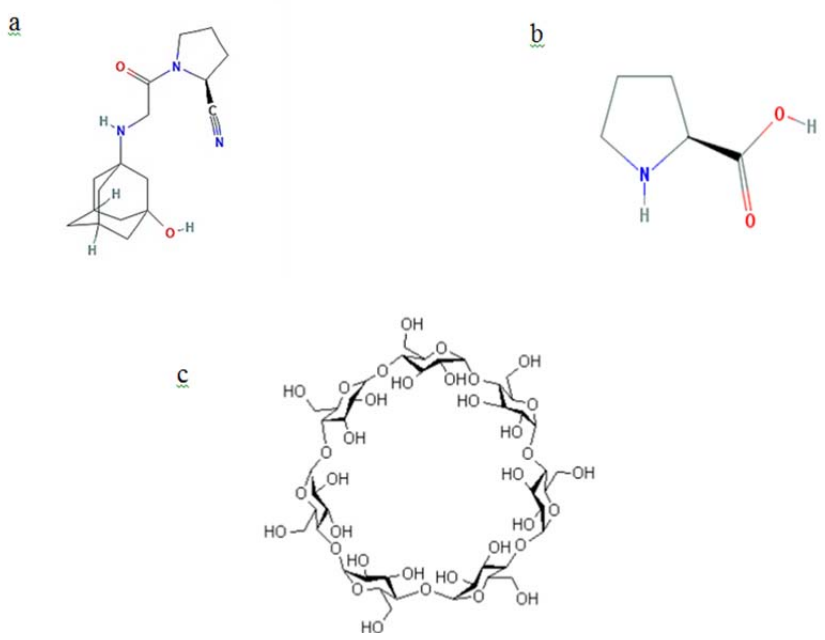

Fig. 2. Chemical structure of a) vildagliptin; b) L-proline and c) 8-hydroxy β cyclodextrin

While intermolecular interaction between the host and guest molecules (hydrogen bonds, hydrophobic interactions and van der Waals forces) contributed to cooperative binding processed when synthetic cyclodextrin were used [31]. The electrochemical performance characteristics of the proposed (HPβCD) sensor were systematically evaluated in Table 1 according to IUPAC standards [24]. The slope of the calibration plots is 55.3 and 43.6 mV/decade for ionophore based sensor and ionophore free sensor, respectively. Close Nernstian slope to theoretical value of monovalent positive ion (60 mV/decade) is attributed to improve selective inclusion complexation and complementary ionic or hydrogen bonding of host molecule, which recognize the structure of guest molecule [32].

Table 1. Comparison between vildagliptin sensors in presence and absence of β-cyclodextrin

parameter	**Ionophore based sensor**	**Ionophore free sensor**
Slope (mV/decade)	55.3	43.6
Intercept (mV)	425.7	175.9
LOD (mol.L^{-1})	5×10^{-6}	7.5×10^{-5}
Response time (sec.)	15	15
Working pH range	4-8	4-8
Concentration range (mol.L^{-1})	1×10^{-5} to 1×10^{-1}	1×10^{-4} to 1×10^{-1}
Stability (days)	15	15
Correlation coefficient	0.9993	0.9427

Moreover, the ionophore based sensor displayed relatively constant calibration slopes for day to day measurements ±3 mV/decade over a period of 3 days. Fig. 3 shows a linear relationship between measured potential (E) and the corresponding logarithmic concentrations of vildagliptin (C) showing wide range for ionophore based sensor. Detection limit is also decreased by about one order of magnitude for ionophore based sensor.

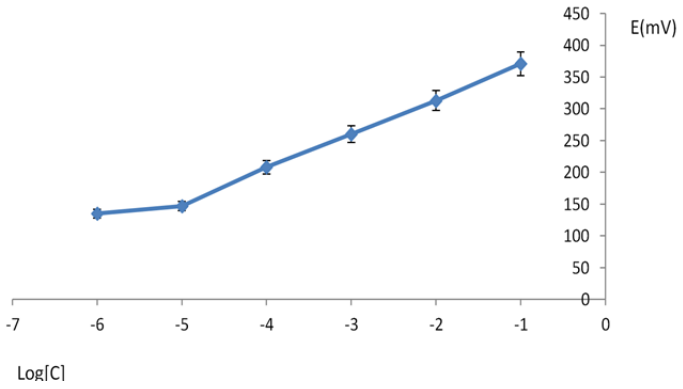

Fig. 3. Profile of the potential in (mV) versus log concentration of vildagliptin in mol.L^{-1} obtained by using hydroxyl β-cyclodextrin sensor

Enhanced electrochemical response of (HPβCD) sensor suggests the molecular recognition between the functionalized cyclodextrin (host) and VIL (guest) molecules through cooperative binding process [31]. Response time was recorded by increasing vildagliptin concentration by up to 4-fold for both sensors. Almost same time required for the both sensors to reach values within ±1 mV of the final equilibrium potential. The potential pH profile obtained indicates that the response of the (HPβCD) sensors is fairly constant over the pH range 4-8 (Fig. 4). This is attributed to complete ionization of vildagliptin above its pKa~9. Higher potential were encountered at acidic medium (<pH 3) is due to interference of hydronium ion. On the other hand the potential of 10^{-3} vildagliptin solution is decreased at alkaline medium (>pH 9) since the unionized form is predominent. This conterary effect was observed for 10^{-4} vildagliptin probably due to significant interference of sodium ion at higher pH values.

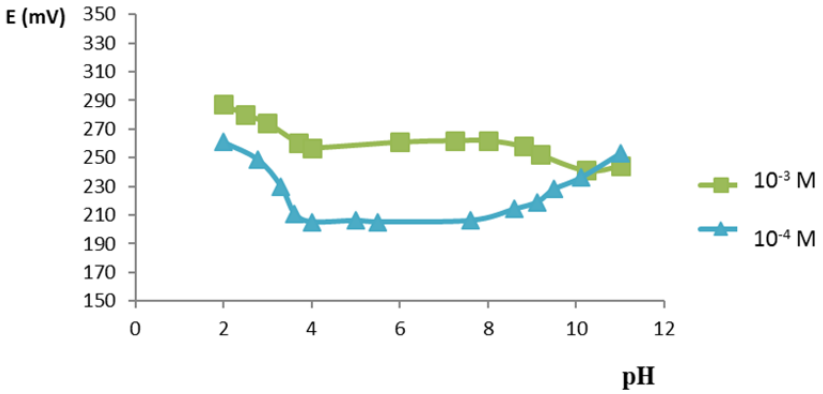

Fig. 4. Effect of pH on the response of the proposed electrode

Sensor selectivity was examined in presence of in-process impurity. Showing subnernstian response, obtained for L-proline using the proposed ionophore based sensor confirms the selectivity of vildagliptin (Fig. 5).

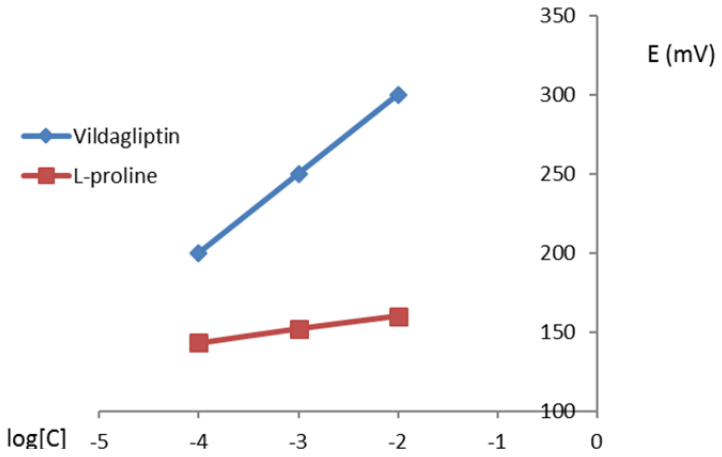

Fig. 5. Chart of L-proline and vildagliptin responses showing sub nernstian slope of L-proline for the proposed ionophore based sensor

Selectivity coefficients of the proposed (HPβCD) sensor in the presence of interfering impurity and inorganic cations (Na^+ and Mg^{2+}) are presented in Table 2.

Table 2. Potentiometric selectivity coefficient (K pot vildagliptin) of the proposed electrode

Interferent	**Selectivity coefficient**
	Ionophore based sensor
Sodium ion	6.5×10^{-3}
Magnesium ion	4.5×10^{-3}
L-proline	1.4×10^{-2}

The results revealed that the proposed membrane sensor displays high selectivity and low response for these interfering species. Synthetic mixtures containing vildagliptin and L-proline in different ratios were also analyzed by the proposed ionophore based sensor. The sensors can be successfully used for selective determination of vildagliptin in presence of up to 90% L-proline, Table 3.

Table 3. Determination of vildagliptin by the proposed electrode in synthetic mixture with L-proline up to 90%

Drug concentration (mol.L^{-1})	**Total concentration (mol.L^{-1})**	**Impurity % (proline)**	**Recovery %**	
0.00001	0.00011	91	101.52	
0.0001	0.0002	50	97.96	
0.001	0.0011	9	99.28	
0.01	0.0101	1	100.99	
			Mean	99.94
			RSD%	1.63

3.1. Effect of pH

For quantitative measurements with ion selective electrodes, studies were carried out to reach the optimum experimental conditions. Vildagliptin solution of 10^{-1} mol.L^{-1} was adjusted to pH 7 with 0.1 M HCl. The potential pH profile obtained indicates that the response of the (HPβCD) sensor is fairly constant over the pH range 4–8 (Fig. 4). The presented figure showed higher potentials at acidic (<pH 3) and alkaline (>pH 9) media that may be due to significant interference of hydronium or sodium ions, respectively.

3.2. Potentiometric determination of vildagliptin in pharmaceutical formulation (Galvus®)

The proposed (HPβCD) sensor was applied for the determination of vildagliptin in Galvus® tablets. The recovered results prove the applicability of hydroxyl propyl β-cyclodextrin sensor for the determination of vildagliptin in Galvus® tablets without interference from the common tablet excipients namely lactose anhydrous, microcrystalline cellulose, sodium starch glycolate and magnesium stearate. Concentrations of vildagliptin were calculated using the corresponding regression equation and the data were presented in Table 4.

Table 4. Determination of vildagliptin in pharmaceutical dosage form by the proposed electrode

Tablet concentration (mol/L)[a]	Recovery %
1×10^{-2}	103.71
1×10^{-4}	102.30
Mean	103.00
SD	0.997
RSD	0.967

[a] the corresponding concentration of tablet is 50 mg/tablet

To examine the validity of hydroxypropyl β-cyclodextrin sensor, the results were compared to those of the manufacturer's specification reversed phase HPLC method and no significant difference was observed as shown in Table 5.

Table 5. Statistical comparison for the results obtained by the proposed electrode and the reported method for the analysis of vildagliptin in bulk powder form

Item	Ionophore based sensor	Manufacturer's method[a]
Mean	99.07	99.397
SD	0.740	0.393
SE	0.332	0.131
Variance	0.55	0.155
n	5	9
Student's t-test	0.899 (2.18)	
F value	3.532 (6.04)	

[a]Manufacturer's method is a gradient HPLC method using ammonium di-hydrogen orthophosphate (solution A) and acetonitrile (solution B) as a mobile phase at pH 6.8 adjusted with ortho-phosphoric acid on C18 column at 210 nm

-Figures between parentheses are the corresponding theoretical values of t and F at a 0.05 level of significance.

4. CONCLUSION

The investigated potentiometric method using hydroxypropyl β-cyclodextrin (HPβCD) as ionophore was found to be simple, rapid, low cost and more sensitive than the HPLC manufacturer's method. The described ionophore based sensor is sufficiently selective for the quantitative determination of vildagliptin in drug substance and pharmaceutical product that can be successfully applied in presence of tablet excipients without previous treatments. Moreover, the potentiometric determination of vildagliptin was successfully applied for the analysis of synthetic laboratory mixture with L-proline; it's in process impurity. The better response characteristics and selectivity coefficient of hydroxypropyl β-cyclodextrin sensor

and low detection limit offer accurate determination of vildagliptin in low concentrations than the ionophore free sensor. The validated potentiometric method offers a simple analytical tool for online drug monitoring during its synthesis process and can be used for routine analysis of vildagliptin in quality control laboratories.

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