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A Selective Sensor for Determination of Sitagliptin phosphate in Pharmaceutical Formulation

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Abstract- A selective electrode was developed for determination of sitagliptin using precipitation based technique with ammonium reineckate as anionic exchanger in polyvinyl chloride matrix. Linear responses of 1×10^{-5} to 1×10^{-2} M with slope of 40.9 mV/decade within pH 4-7. The percentage recovery for determination of sitagliptin by the proposed sitagliptin selective electrode was 100.06±1.15. Determination of sitagliptin in its pharmaceutical formulation by the proposed electrode revealed its applicability for its determination. The proposed method was compared with a reported one. No significant difference for both accuracy and precision was observed. The electrode exhibit good selectivity for sitagliptin with respect to a large number of inorganic, organic cations, sugars and amino acids. The proposed electrode offers the advantages of simplicity, accuracy and applicability to turbid and colored samples. The fabricated sensor was validated according to the International Conference on Harmonization (ICH) guidelines and successfully applied for the determination of the studied drug in pure form and pharmaceutical formulation without any interference.

Keywords- Ammonium Reineckate, Sitagliptin, Potentiometry, Poly Vinyl Chloride

1. INTRODUCTION

Sitagliptin phosphate (STA) is [(R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4] triazolo [4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine] phosphate monohydrate, (Fig. 1). It is an orally active and selective inhibitor of dipeptidyl peptidase-IV that is used for treatment of type II diabetes [1]

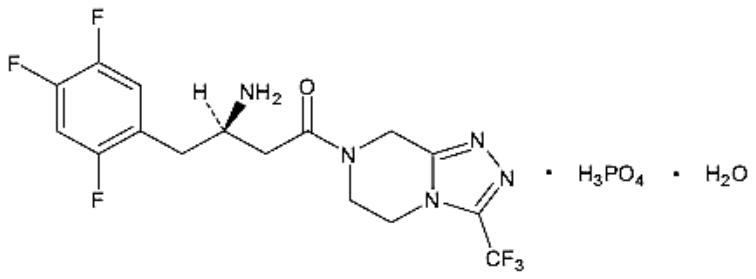

Fig. 1. Chemical structure of sitagliptin phosphate

The literature survey reveals several analytical methods for quantitative estimation of sitagliptin by spectrophotometric methods [2,3], high performance liquid chromatographic method [4], selective sensors using β-cyclodextrin or calix-8-arenein were investigated [5], in addition to its determination in combinations with other drugs [6-10].

Modern ion selective electrodes based on material transport across a specific membrane are now widely used in the determination of trace amounts of analytes. The material transport includes either neutral and charged complex species or simple ions. The high selectivity of these electrodes imparts a great advantage over other techniques [11]. Analytes which are in colored, turbid or viscous samples can be determined accurately.

They show rapid response to changes in the concentration. Furthermore, they may be used for measurement over a wide concentration range. Ion selective electrodes (ISEs) are generally tolerant of small changes in pH. A further advantage is that they are relatively simple and cheap to develop, set up and run. Moreover, the chemical design of the electrodes has been developed to give superior selectivity and response [12].

Although chromatographic methods are highly selective, however, their requirements of cleaning up samples and sophisticated instrument preclude their use in routine analysis.

In spite of progress in the design of highly selective electrodes for various ions, there has not been any report on the development of selective and sensitive Sitagliptin sensors. This paper describes the construction, potentiometric characterization, and analytical application of sitagliptin sensor.

Precipitation-based technique of STA with ammonium reineckate (NH_4RNC) was used in the preparation of the sensor. The investigated potentiometric method was found to be

simple, rapid, low cost, more sensitive than the reported methods, and can be accurately and successfully applied for the determination of sitagliptin in pure forms and in pharmaceutical formulation without previous treatment.

2. EXPERIMENTAL

2.1. Instruments

•Jenway digital ion analyzer model 3330 (UK) with Ag/AgCl double junction reference electrode no. 924017-LO3-Q11C.
• Bandelin Sonorox, Rx 510 S, magnetic stirrer (Hungarian)
• pH glass electrode Jenway (UK) no. 924005-BO3-Q11C.

2.2. Materials

2.2.1. Reference sample

Standard sitagliptin phosphate powder (STA) was kindly donated by Merck (Whitehouse station, NJ). Its purity was found to be 98.87±0.95% (n=6), according to a reported spectrophotometric method [2].

2.2.2. Pharmaceutical formulation

Januvia® tablets BN: Y001092, labeled to contain 100 mg Sitagliptin.

2.3. Reagents

All chemicals and reagents used throughout this work were of analytical grade (water used was bi-distilled):

- Ammonium Reineckate (NH_4RNC), $NH_4[Co(NH_3)_2(SCN)_4]$, freshly prepared aqueous solution; Aldrich
- Dioctyl phthalate (DOP); Sigma.
- Poly (vinyl chloride) (PVC) of high molecular weight; Fluka Chemie GmbH, Germany.
- Sodium hydroxide, hydrochloric acid and potassium chloride; Prolabo.
- Tetrahydrofuran (THF); BDH

  Aqueous solution of ammonium reinckate in concentration of 1.0×10^{-2} M was prepared.

2.4. Standard solutions

2.4.1. Sitagliptin stock solution (1×10^{-1} M)

It was freshly prepared daily by transferring 5.233 g of STA into 100 ml volumetric flask and dissolving in distilled water and tightly closed.

2.4.2. Sitagliptin working solutions (1×10^{-7} to 1×10^{-2} M)

They were freshly prepared by suitable dilution from their stock solution using distilled water and kept in well-closed tight container to avoid air oxidation.

2.5. Procedures

2.5.1. Precipitation based technique for the preparation of PVC-membrane sensor

A 50 ml aliquot of 1.0×10^{-2} M aqueous STA solution was mixed with 50 ml of aqueous 1.0×10^{-2} M aqueous NH_4RNC.

The resultant precipitates formed was filtered using Whitman no. 42 paper, washed with cold water, dried at room temperature (about 20 ^{o}C) and grinded to fine powder. Elemental analysis of the formed complex confirmed the formation of drug: ion exchange in a ratio of 1:1.

In a glass Petri dish (5 cm diameter), 10 mg of STA-ion exchange was thoroughly mixed with 0.35 ml of DOP and 0.19 g of PVC. The mixture was dissolved in 5 ml of THF.

The Petri dish was covered with a filter paper and left to stand overnight to allow solvent evaporation at room temperature. A master membrane with a thickness of 0.1mm was obtained; a disk (≈8 mm diameter) was cut using a cork borer and pasted using THF, to an interchangeable PVC tip that was clipped into the end of the electrode glass body.

Equal volumes of 10^{-2} M STA and 10^{-2} M KCl was mixed and this solution was used as an internal reference solution. Ag/AgCl wire (1 mm diameter) was immersed in the internal reference solution as an internal reference electrode.

The sensor was conditioned by soaking in 10^{-2} M aqueous drug solution for 24 h. Storage was in the same solution when not in use.

2.5.2. Direct potentiometric determination of STA in its pure sample

The conditioned sensor was calibrated by separately transferring 50 ml aliquots of solutions covering the concentration range of (1×10^{-7} to 1×10^{-2} M) STA, into a series of 100 ml beakers. The electrode system was immersed in each solution, with constant stirring in conjunction with a Jenway reference electrode. The electrode potential was plotted versus each negative logarithmic concentration of STA. The calibration plot obtained was used for subsequent measurements of unknown samples.

2.5.3. Direct potentiometric determination of STA in its pharmaceutical formulation

Ten tablets were weighed and powdered. An amount of the powdered tablets equivalent to 0.5233 g STA was transferred into a 100 ml volumetric flask. The volume was completed with water to prepare a 10^{-2} M aqueous solution of STA .Suitable dilutions were performed using distilled water to obtain solutions of 10^{-3}&10^{-4} M of STA. The procedure was then completed as under section 2.

3. RESULTS AND DISCUSSION

Selective membranes in ion selective electrodes (ISEs) have shown both ion exchange and perm-selectivity of the sensor ions [13]. The fabricated membrane suggested in this section, belong to the type of simple supported ion exchange with PVC as a polymer matrix.

Ammonium reinickate was reported as famous anionic exchanger [14,15] it has been used in the formation of many sensors [16,17]

The present study originates from the fact that STA possesses cationic center. This fact suggests the use of ion exchangers of the anionic type like, NH_4RNC for its low solubility product and suitable grain size.

STA was found to form 1:1 ion association complex with NH_4RNC, as proved by elemental analysis of STA- NH_4RNC.

3.1. Sensors fabrication

The proposed sensor was prepared and electrochemically evaluated as prospective sensor for STA according to the IUPAC guidelines [18] (Table 1).

It has been reported that PVC matrix is a regular support and reproducible trap for the sensed ions [19]. It has the advantages of chemical inertness, high tensile strength and low cost, but its use make a need for a plasticizer [20]. In the present investigation, dioctylphthalate was chosen as plasticizer. The membrane constituents were dissolved in THF that was slowly evaporated at room temperature leading to membrane formation.

Table 1. Electrochemical response characteristics of the investigated STA electrode

Parameter	STA-RNC
Slope (mV/ decade) *	40.9
Intercept (mV)	260.9
LOD (M)	2.0×10^{-6}
Response Time (Sec.)	30
Working pH Range	4-7
Concentration Range (M)	10^{-5}-10^{-2}
Stability (days)	30
Accuracy (mean ±SD)*	100.06±1.15
Correlation coefficient	0.9994

*Average of three determinations

In the present study, DOP has been used in the fabrication of the proposed sensor. It plasticized the membrane and adjusted both permittivity of the final organic membrane and mobility of the ion exchanger sites.

With PVC, the diesters of carboxylic acids (DOP) were found to be the optimum plasticizers. It dissolves the ion association complexes and adjusts both of the membrane permittivity and ion exchange sites mobility to give highest possible selectivity and sensitivity [21,22]. Such adjustments influence the partition coefficient of the studied ion association complex with subsequent effect on electrode selectivity.

The membrane constituents were dissolved in THF that was slowly evaporated at room temperature leading to membrane formation.

The electrochemical cell of the suggested membrane electrodes for the determination of STA can be illustrated s followed:

Double junction Ag/AgCl Reference Electrode	Test Solution	Sitagliptin Association Complex Incorpotated in PVC matrix	Internal reference Solution: 10^{-2} M KCl+10^{-2} M Sitagliptin in 1:1 ratio	Ag/AgCl Internal Reference wire

3.2. Sensors calibration and response time

The potential displayed by the proposed electrode for constructive measurements of standard drug solution in the same day and from day-to-day did not vary by more than ±3 mV. Calibration slopes did not change by more than ±3 mV/decade concentration over a period of 30 days. The required time for the electrodes to reach values within ±1 mV of the final equilibrium potential after increasing the drug concentration 10-folds was found to be 30 s. The slope of the calibration plot was 40.9 mV/concentration decade for STA-RNC (Fig. 2).

Slope value of the electrode was about 40 mV; Deviation from the ideal Nernstian slope (60 mV) for the sensor stems from the fact that the electrode responds to the activities of drug anion rather than its concentration. The potentiometric responses of the STA-selective electrode at the optimum pH range and temperature were linear over a drug concentration range of 10^{-5}-10^{-2} M. The investigated electrode exhibit fast response time (30 s) and fair stability (30 days). The response characteristics of the electrode are summarized in (Table 1). The proposed method was compared with a reported one [2], no significant difference was observed (Table 2).

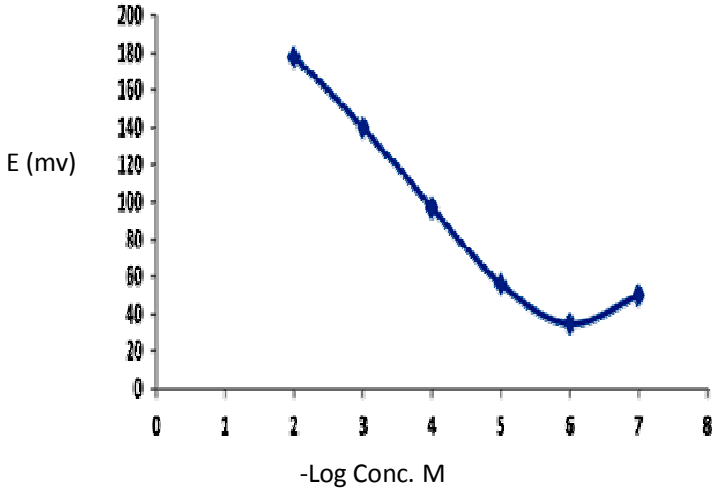

Fig. 2. Profile of the potential in mV *vs.* –log concentration of sitagliptin phosphate obtained by using the proposed electrode

Table 2. Statistical comparison for the results obtained by the proposed electrode and the reported method for the analysis of sitagliptin phosphate in pure form

Parameter	STA-RNC	Reported method[2]
Mean	100.06	99.87
S.D.	1.15	0.95
N	4	6
Variance	1.32	0.91
Student's t-test	0.273 (2.306)*	
F-value	1.45 (5.41)*	

*The values in parentheses are the corresponding theoretical values for t and F at P=0.05

3.3. Sensors pH and temperature

For quantitative measurements with ISEs, studies were carried out to reach the optimum experimental conditions. The effect of pH on the response of the investigated electrode using 1×10^{-4} and 1×10^{-3} M solutions of STA for the electrodes was studied. It was apparent from the potential–pH profile that the responses are fairly constant over the pH range 4-7, i.e., in this pH range STA is completely ionized, dissociated and sensed. Above pH 7, the potential showed a sharp decrease due to the formation of nonprotonated primary amino group of STA.

Below pH 4, the electrode response increased with the increase of analyte acidity; the membrane may extract H^+, leading to noisy responses (Fig. 3).

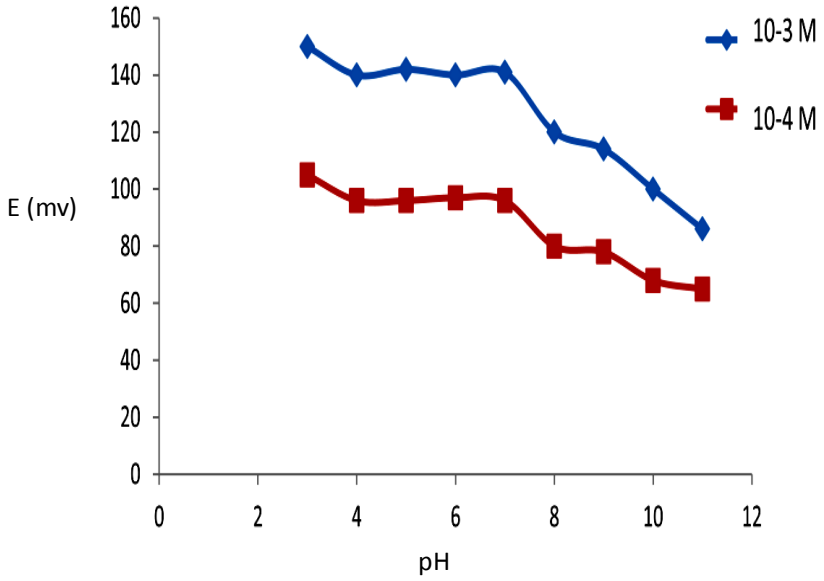

Fig. 3. Effect of pH on the response of the proposed electrode

Upon studying the effect of temperature, the suggested electrode exhibit slight increase in its potential as the temperature rises in the range of 25-35 ^oC, however, the calibration graphs obtained at different temperatures were parallel. The limit of detection, slope and response time did not significantly vary with variation of temperature, indicating reasonable thermal stability of PVC membrane up to 35 ^oC.

3.4. Sensors selectivity

The effect of interfering substances upon the performance of the sensors was studied by separate solution method using the following equation:

$$-\log (K^{Pot}_{\text{primary ion, interferent}}) = [f\frac{(ESTA - EM)}{2.303\ RT/ZSTAF}]+[1 - \frac{ZSTA}{ZM}]\log C_{STA}$$

where *E*STA is the potential measured in 10^{-3} M STA solution, *E*M the potential measured in 10^{-3} M interferent solution, *Z*STA and *Z*M are the charges of STA and interfering ion, respectively, and 2.303*RT*/*Z*STA*F* represents the slope of the investigated sensor (mV/concentration decade).

Table 3, shows the selectivity of the proposed sensors in the presence of the related substances that always present with STA in the dosage formulation.

The proposed sensor was accurately applicable for the determination of STA in Janovia® tablets. The recovery was found to be 99.70±1.11. Placebo experiments contain all additives in the same ratio as that used in tablets were investigated. Talc, Cellulose, magnesium stearate, titanium dioxide and sodium stearyl fumarate which present in the tablet did not show any interference. Thus, analysis was carried out without prior treatment or extraction. The proposed STA-selective sensors have shown excellent selectivity.

Table 3. Potentiometric selectivity coefficients ($K^{pot}_{sitagliptin\ I}$) of the proposed electrode

Interferent**	Selectivity coefficients*
Glucose	1.3×10^{-3}
KCl	1.84×10^{-2}
NH_4Cl	2.44×10^{-2}
$CaCl_2$	8.44×10^{-3}
Starch	2.18×10^{-3}
NaCl	1.31×10^{-3}
Talc	0.83×10^{-3}
Lactose	1.94×10^{-4}
Urea	1.73×10^{-3}
Metformin	1.97×10^{-3}

* Average of 3 determinations

**All interferents are in the form of 1×10^{-3} M solution

4. CONCLUSION

The described novel sensor was sufficiently simple, selective and rapid tools that can be used for the quantitative determination of sitagliptin in pure form and pharmaceutical formulation. It offers advantages of fast response and elimination of drug pretreatment or separation steps. It can be used for routine analysis of sitagliptin in quality control laboratories.

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