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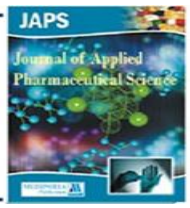

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Comparative Study of Beta Cyclodextrin and Calix-8-Arene as Ionophores in Potentiometric Ion-Selective Electrodes for Sitagliptin Phosphate

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ABSTRACT

Two novel Sitagliptin (STG) selective electrodes were investigated with di-octyl phthalate as a plasticizer in a polymeric matrix of polyvinyl chloride (PVC). Sensor 1 was fabricated using β-cyclodextrin, while sensor 2 was constructed using calix-8-arene as ionophores. Linear responses of STG within the concentration ranges of 10−7 to 10−2, and 10−8 to 10−2 mol L−1 were obtained using sensors 1 and 2, respectively. Nernstian slopes of 58.57 and 59.88 mV/decade over the pH range of 5-6.5 were observed. The selectivity coefficients of the developed sensors indicated excellent selectivity for STG. The proposed sensors displayed useful analytical characteristics for the determination of STG in bulk powder, different pharmaceutical formulations, and biological fluids (plasma and urine).The two novel electrodes offer the advantage of determination of STG in biological fluids without pretreatment which is convenient for monitoring STG levels in clinical studies.

Keywords: Sitagliptin; beta-cyclodextrin; calix-8-arene; electrode.

INTRODUCTION

Sitagliptin (STG), [(2R)-1-(2,4,5-trifluorophenyl)-4-oxo-4-[3-(trifluoromethyl)- 5,6 dihydro [1,2,4] triazolo [4,3-a]pyrazin-7(8H)-yl] butan-2-amine] (Fig. 1a) and meformin (MET), N,N-dimethylimidodicarbonimidic diamide (Fig. 1b) are two well known hypoglycemic drugs. STG is a novel oral hypoglycemic drug of the dipeptidyl peptidase 4 inhibitor class (Sekaran & Rani, 2010) that represents a new therapeutic tactic to the treatment of type 2 diabetes that acts to stimulate glucose-dependent insulin release and reduce glucagon levels. STG is used as a single therapy or in combination with MET that belongs to the biguanide groups (Ray, 1961). In STG structure, the substituted bicyclic piperazine replacements improves metabolism of metabolically labile piperazine ring, whereas adjustments of the fluorine substituents provides increased potency (Kim *et al.*, 2005).

Few methods have been described for the determination of STG in pharmaceutical preparations or biological fluids including spectrophotometry (Sekaran & Rani, 2010) (El-Bagary *et al*., 2011) and HPLC (Nirogi *et al.,* 2008; Zeng *et al.,* 2008; Zeng *et al.,* 2010; El-Bagary *et al*., 2011). Besides, some methods have been reported for determination of STG in presence of MET including spectrophotometry (El-Bagary *et al.,* 2011) and HPLC (El-Bagary *et al.,* 2011).

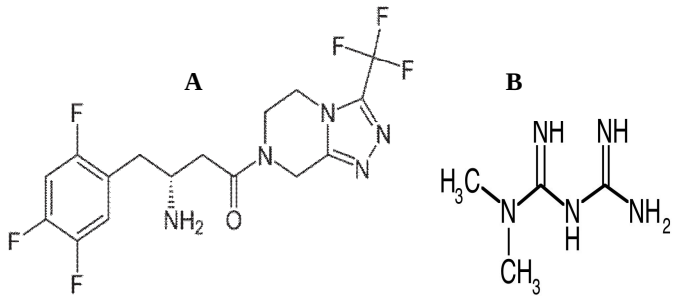

Fig. 1: Chemical structure (a) of Sitagliptin molecule and (b) of Metformin molecule.

Cyclodextrins are known to accommodate a wide variety of organic, inorganic and biologic guest molecules to form stable host–guest inclusion complexes or nanostructure supramolecular assemblies in their hydrophobic cavity while exhibiting high molecular selectivity and enantioselectivity (El-Kosasy *et al.,* 2011; Staden & Nejem, 2006).

They have been previously applied as sensor ionophores in potentiometric ion selective electrodes for the determination of fluorinated surfactants(Patil *et al.,* 2007), chiral molecules incorporating aryl rings (Ozoemena & Stefan,2005), protonated amines (El-Kosasy,2003) and quaternary ammonium drugs (El-Kosasy *et al.,* 2009). Calixarenes are cavity-shaped cyclic oligomers made up of phenol units linked via alkylidene groups. Their configuration includes a number of selective factors, such as cavity-size, conformation and substituents, which leads to the formation of typical host–guest complexes with numerous compounds and allow for a variety of applications in ion-selective membranes and electrodes (Park *et al.,* 2001; Kumar & Shim, 2009; Kivlehan *et al.,* 2007).

The present work describes the use of β-cyclodextrin and calix -8- arene as neutral ionophores for the development of novel sensors for the determination of STG in bulk powder, different pharmaceutical formulations, biological fluids (plasma and urine), and in presence of MET.

EXPERIMENTAL

Apparatus

A Jenway pH meter 3310 pH /mV /˚C meter with Orion, reference electrode (Ag/Agcl, double junction) model 63178 USA 314-771-5750, A Jenco digital ion analyzer model 6209 were used for potential measurements. Jenway pH glass electrode (UK) and Bandelin Sonorox, Rx 510 S, magnetic stirrer (Budapest, Hungary) were used for pH adjustment.

Chemicals and reagents

Des fluoro Sitagliptin MK-0726, 100%, was obtained from Merck Sharp and Dohme Co. (USA). Januvia® tablets (nominally containing 128.5mg of sitagliptin phosphate monohydrate per tablet, Merck Sharp and Dohme Co., Cairo, Egypt) and Janumet® tablets (nominally containing 64.25mg of sitagliptin phosphate monohydrate and 1000mg of metformin per tablet, Merck Sharp and Dohme Co., Cairo, Egypt) were used in this work.

All chemicals and reagents used were of analytical reagent grade, and water was bi-distilled. Polyvinyl chloride carboxylate (PVC carboxylate), β-cyclodextrin (β-CD) and calix-8-arene were obtained from Fluka Chemie Gmbh (Steinheim, Germany). Di-octyl phthalate (DOP) was purchased from Aldrich (Steinheim, Germany). Tetrahydrofuran (THF) was obtained from Merck (Dermstadt, Germany).

Potassium chloride, ammonium sulphate, sodium hydroxide and hydrochloric acid were obtained from Prolabo (Pennsylvania, USA). Plasma and urine were supplied by VACSERA (Giza, Egypt) and used within 24 h.

Procedures

Fabrication of membrane sensors

In a 5-cm Petri dish, 400 mg β-CD or 400 mg calix-8-arene was mixed with 190 mg PVC carboxylate, dissolved in 0.35 ml DOP, then thoroughly mixed with 5 ml THF till complete homogeneity for the preparation of sensor 1 & 2, respectively. The Petri dishes were covered with filter paper and left to stand overnight at room temperature to allow solvent evaporation.

Master membranes 0.1 mm in thickness were obtained. The coated graphite electrodes were constructed using a graphite bar 2.5 cm in length (about 3mm in diameter).

One end of the bar was used for connection while the other, about 1 cm in length, was dipped in the electro active membrane mixture. The process was repeated several times until a layer of proper thickness was formed covering the terminal of the graphite bar. The electrodes were left standing at room temperature overnight till completely dried. The uncoated end of the graphite rod was sealed in a poly tetra ethylene tube; the tube was filled with metallic mercury into which a copper wire (about 1 mm in diameter) was dipped. The sensors were conditioned by soaking in 10−2 mol L−1 aqueous STG solution for 24 h, and they were stored in the same solution when not in use.

Sensors calibration

The conditioned sensors were calibrated by separately transferring 50 mL aliquots of solutions (10−8to 10−2 mol L−1) of STG into a series of 100-mL beakers. The membrane sensors, in conjunction Aldrich reference electrode, were immersed in the above test solutions and allowed to equilibrate while stirring.

The potential was recorded after stabilizing to ±1 mV and the electromotive force was plotted as a function of the negative logarithm of STG concentration. The sensors were washed in distilled water between measurements.

Effect of pH

The effect of pH on the response of the investigated electrodes was studied using 10−3 and 10−4 mol L−1 solutions of STG with pH ranging from 2 to 10(while adjusting pH using 0.1N NaOH and 0.1 N HCl)

Sensors selectivity

The potentiometric selectivity coefficients (Kpot A.B) of the proposed sensors towards different substances were determined by a separate solution method using the following equation (IUPAC, Analytical Chemistry Division, Commission on Analytical Nomenclature, 2000):

$$-\log (K_{pot}A.B) = E1 - E2/(2.303\ RT/Z_AF) + (1 - Z_A/Z_B)\log \alpha A$$

where KpotA.B is the potentiometric selectivity coefficient, E1 is the potential measured in 10−3 mol L−1 STG solution, E2 is the potential measured in 10−3 mol L−1 interferent solution, ZA and ZB are the charges of STG and interfering ion, respectively, αA is the activity of the drug and 2.303RT/ZAF represents the slope of the investigated sensors (mV/concentration decade).

Determination of STG in pharmaceutical preparations

A portion of Januvia® and Janumet® tablets powder equivalent to 0.26971 g STG were transferred separately into two 50-mL volumetric flasks and filled to the mark with bi-distilled water to prepare 1.0 x 10^{-2} mol L−1 stock solution of each. Then a serial dilution was made from the prepared stocks to obtain 1.0 x $10^{-3,}$ 1.0 x 10^{-4} and 1.0 x 10^{-6} mol L−1 samples of each of Januvia® and Janumet®. The potentiometric measurements of the prepared samples were performed using the proposed sensors in conjunction with Aldrich reference electrode, and the potential readings were compared to the calibration plots.

Determination of STG in plasma

One milliliter of each of 10−2, 10−3 and 10−5 mol L−1 standard drug solution were added separately into three 20-mL stoppered shaking tubes each containing 9 mL of plasma and the tubes were shaken for 1 min. The membrane sensors were immersed in conjunction with the reference electrode in these solutions and then washed with water between measurements. The emf produced for each solution was measured by the proposed sensors, and the concentration of STG was obtained from the corresponding regression equation.

Determination of STG in urine

One milliliter of each of 10−2, 10−3 and 10−5 mol L−1 standard drug solution were added separately into three 20-mL stoppered shaking tubes each containing 9 mL of urine and the tubes were shaken for 1 min. The membrane sensors were immersed in conjunction with the reference electrode in these solutions and then washed with water between measurements. The emf produced for each solution was measured by the proposed sensors, and the concentration of STG was obtained from the corresponding regression equation.

RESULTS AND DISCUSSION

The molecular recognition and inclusion complexation are of current interest in host–guest and supramolecular chemistry and offer a promising approach to chemical sensing (Mittal *et al.*, 2007)(Zanganeh & Amini,2008). The use of selective inclusion complexation and complementary ionic or hydrogen bonding are two main strategies for preparing synthetic host molecules, which recognize the structure of guest molecules (Gorski *et al.*, 2010).

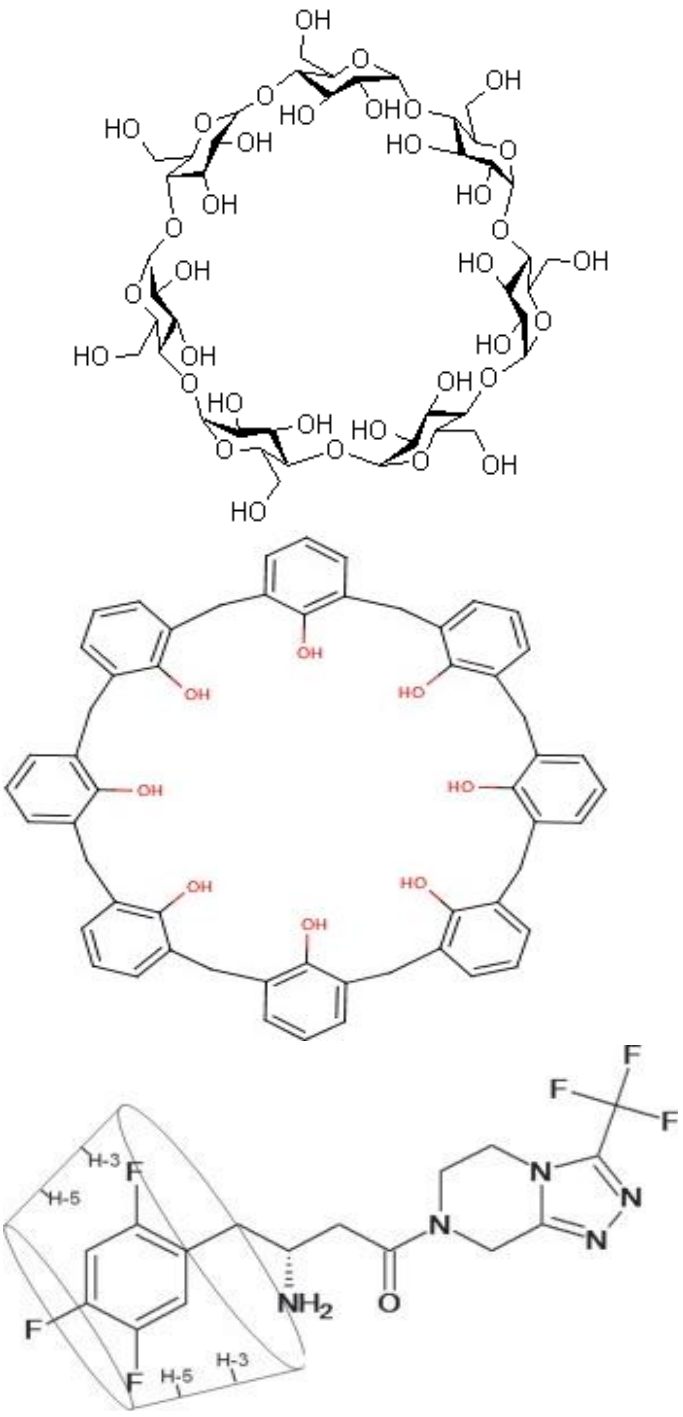

Fig. 2: Chemical structure (a) of β -cyclodextrin molecule,(b) of calix-8-arene molecule, and (c) the proposed structure for the STG–CD inclusion complex.

Modified cyclodextrins (CDs), either natural or synthetic, are viewed as molecular receptors, as is shown in Fig. 2. In the case of natural CD, cooperative binding with certain guest molecules was mostly attributed to intermolecular hydrogen bonding between the CD molecules, while intermolecular interactions between the host and guest molecules (hydrogen bonds, hydrophobic interactions and Van der Waals forces) contributed to cooperative binding processes when synthetic CDs were used (Sideris, 1999). Although the size and geometry of the guest mainly govern the binding strength, it is possible to influence the host–guest interactions by modifying the three hydroxyl groups on each glucose unit. Indeed, the use of 2-hydroxy propyl β-cyclodextrin enhanced the interaction properties between host and guest molecules (Flink *et al.*, 2000).

Calixarenes are well-known as selective ligands for various ions through dipole–dipole interactions, as shown in Fig. 2. They can complex with a large variety of cation substrates to form stable host–guest inclusion complexes. This property of calixarenes has been largely exploited for the development of a number of cation selective electrodes (Bakker & Qin, 2006; Chen *et al.*, 2006; Zareh & Malinowska, 2007).

STG is a[(2R)-1-(2,4,5-trifluorophenyl)-4-oxo-4-[3-(trifluoromethyl)- 5,6 dihydro [1,2,4] triazolo [4,3-a]pyrazin-7(8H)-yl] butan-2-amine] having a pka of 7.7(European Medicines Agency, London, 2009). ROESY NMR experiments were carried out on samples containing (R)-STG and β-CD to explore the structure of the inclusion complexes. The cross-peaks between CD and STG signals indicate the spatial proximity of the protons. Cross-peaks between aromatic STG and inner CD protons can be observed in each spectrum. The most intensive cross-peaks were observed between the aromatic STG protons (H-13 and H-16) and the inner CD protons (3-H, 5-H). Less intensive cross-peaks between H-10 STG and 3-H CD protons provided additional information about the direction of inclusion and confirmed the involvement of the aromatic ring in the complexation. These data suggested that the trifluorobenzene moiety of the guest gets in the CD cavity from the wider secondary rim (Sohajda *et al.*, 2011). The proposed structure for the STG–CD complex is shown in Fig.2 (c).

The present work evaluates the possibility of using β-cyclodextrin and Calyx-8-arene as sensor ionophores in the preparation of STG-selective electrodes 1 and 2, respectively, using PVC as a polymeric matrix to immobilize the sensors and to attain the formation of highly stable complexes.

Performance and characteristics of STG sensors

The electrochemical performance characteristics of the proposed sensors were systemically evaluated according to IUPAC standards (IUPAC, Analytical Chemistry Division, Commission on Analytical Nomenclature, 2000). Table 1 shows electrochemical response characteristics of the two investigated sensors. Typical calibration plots are shown in Fig.3.

The slopes of the calibration plots are 58.571 and 59.875 mV/concentration decade for sensors 1, and 2, respectively. Deviation from the ideal Nernstian slope (60 mV) is due to the electrodes responding to the activity of the drug cation rather than its concentration. The sensors displayed constant potential readings for day to day measurements, and the calibration slopes did not change by more than ±2 mV/decade over a period of 23 and 31 days for sensors 1 and2 respectively. The detection limits of the two sensors were estimated according to the IUPAC definition (IUPAC, Analytical Chemistry Division, Commission on Analytical Nomenclature, 2000). Table 1shows that sensor 2 can detect STG in very dilute solutions down to 1.121 ×10−8 mol L−1,while sensor 1 can only detect STG down to 1.01×10−7 mol L−1.

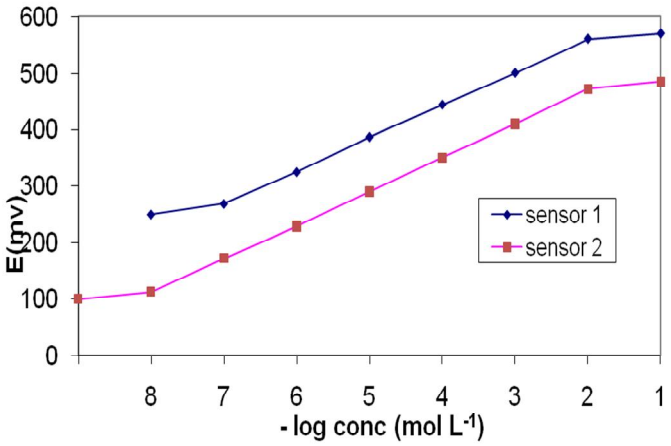

Fig. 3: Profile of the potential in mv versus - log concentration of sitagliptin in mol L-1 obtained by using the sensor 1 & 2.

Table. 1: Electrochemical response characteristics of the two investigated STG sensors.

Parameter	Sensor1	Sensor2
Slope(mV/decade)[a]	58.571	59.875
Intercept(mV)	677.9	590.33
LOD(mol L-1)b	1.01x 10^{-7}	1.121x10-8
Response time(sec.)	40	30
Working pH range	5-6.5	5-6.5
Concentration Range (mol L-1)	1x10^{-7} to 1x10^{-2}	1x10^{-8} to 1x10^{-2}
Stability(days)	23	31
Correlation Coefficient	0.9999	0.99999

[a] *Average of three determinations*
[b] *Limit of Detection*

To examine the validity of the proposed sensors, the obtained results were compared to those of the official method(Pathade *et al.*, 2011) and no significant difference was observed as shown in Table 2.

Table. 2: Statistical comparison for the results obtained by the proposed electrodes and the official method (Pathade *et al.*, 2011) for the analysis of sitagliptin in pure powder form.

Item	Sensor 1	Sensor 2	Official method[b]
Mean[a]	99.88	99.95	99.59
S.D[a]	0.55	0.61	0.69
Variance	0.31	0.37	0.47
n	6	7	10
Student's t-test[c]	0.896(2.160)	1.131(2.145)	
F value[c]	1.542(4.772)	1.267(4.099)	

[a] *Average of three determinations*
[b] *spectrophotometric measurement at 267 nm indist.H2O*
[c] *The values in parentheses are the corresponding theoretical values for t and F at P=0.05*

Dynamic response time

Dynamic response time is an important factor for analytical applications of ion-selective electrodes. In this study, practical response time was recorded by increasing STG concentration by up to 9-fold. The required time for the sensors to reach values within ±1 mV of the final equilibrium potential was 45, and 35 s for sensors 1 and 2, respectively.

Effect of pH and temperature

For quantitative measurements with ion selective electrodes, studies were carried out to reach the optimum experimental conditions. The potential pH profile obtained indicates that the responses of the two sensors are fairly constant over the pH range 5–6.5. Therefore, the pH range from 5 to 6.5 was assumed to be the working pH range of the two sensors. The results suggest that the electrodes exhibit a slight increase in their potential as the temperature rises in the range of 25–45 ◦C. However, the calibration plots obtained at different temperatures are parallel, and the limit of detection, slope and response time do not significantly vary with temperature indicating reasonable thermal stability up to 35 ◦C.

Sensors selectivity

Table 3 shows the potentiometric selectivity coefficients of the proposed sensors in the presence of some interfering species and some inorganic cations (K+, Na+,and NH4+) that are usually found in biological fluids. The results reveal that the proposed membrane sensors display high selectivity and that sensors 2 displays higher selectivity and lower response for the potentially interfering species than sensor 1.

Table. 3: Potentiometric selectivity coefficients (Kpot.I) of the two proposed sensors using the separate solutions method (SSM) (IUPAC, Analytical Chemistry Division, Commission on Analytical Nomenclature, 2000).

Interferent	Selectivity coefficient[a]	
	Sensor1	Sensor2
Caffeine	2.8×10^{-4}	3.5×10^{-4}
Pethidine	3.0×10^{-4}	5.0×10^{-4}
Furosemide	1.0×10^{-4}	1.3×10^{-4}
Salicylic acid	3.1×10^{-4}	9.5×10^{-4}
Trisodium citrate	1.4×10^{-5}	2.9×10^{-5}
NiCl2 Hexahydrate	6.6×10^{-3}	5.1×10^{-5}
Citric acid	5.2×10^{-4}	8.6×10^{-4}
$BaCl_2$	1.5×10^{-4}	3.9×10^{-4}
KCl	2.7×10^{-4}	2.3×10^{-4}
NH4Cl	1.8×10^{-5}	1.9×10^{-4}

[a] *Each value is the average of three determinations*
[b] *All interferents are in the form of 1 x 10^{-3} M solution*

Potentiometric determination of STG in pharmaceutical Formulations

The proposed sensors were applied for the analysis of STG pharmaceutical formulations in aqueous solution. The results prove the applicability of the two sensors for the determination of pharmaceutical formulations containing STG alone or in combination with MET. These data are shown in Table 4.

Potentiometric determination of STG in plasma and urine

The results obtained for the determination of STG in spiked human plasma show that a wide concentration range of the drug can be determined by the investigated sensors with high precision and accuracy. The results presented in Table 4 show that sensors 2 is more sensitive than sensor 1 in plasma samples.

For the application to urine, it was found that the two sensors are reliable and give stable results with very good accuracy and high percentage recovery, which is shown in Table 5. The response times of the proposed sensors are rapid (within 45 s), so the sensors are rapidly transferred back and forth between the biological samples and the bi-distilled water between measurements to protect the sensing component from adhering to the surface of some matrix components. It is concluded that the proposed sensors can be successfully applied to in vitro studies and for clinical use without the need for any pretreatment or preliminary extraction procedures from biological fluids.

Table. 4: Determination of Sitagliptin in pharmaceutical dosage formulations by the two proposed electrodes and official method (Pathade *et al.*, 2011).

Pharmaceutical formulation	Recovery(%) ± S.D.[a] of Sitagliptin		
	Sensor1	Sensor2	Official method[b]
Januvia(100mg/tablet)	100.12±0.575	100.17±0.857	99.59±0.69
t-Test[c]	1.312 (2.776)	1.070 (3.182)	
F[c]	1.426 (19.384)	1.557 (4.256)	
Janumet(50mg/tablet)	99.50±0.517	99.85±0.241	99.59±0.69
t-Test[c]	-0.242 (2.776)	0.997 (2.228)	
F[c]	1.763 (19.385)	8.117 (19.385)	

[a] *Average of three determinations*
[b] *spectrophotometric measurement at 267 nm in dist. H2O*
[c] *The values in parentheses are the corresponding theoretical values for t and F at P=0.05*

Table. 5: Determination of Sitagliptin in spiked human plasma by the proposed sensors.

Added(µcg/ml)	Recovery(%) ± S.D.[a]	
	Sensor1	Sensor2
10^{-6} (0.539)	98.82±0.94	100.33±0.91
10^{-7}(0.0539)	99.4±1.38	100.5±1.82

[a] *Average of three determinations*

Table. 6: Determination of Sitagliptin in spiked human urine by the proposed sensors.

Added(µcg/ml)	Recovery(%) ± S.D.[a]	
	Sensor1	Sensor2
10^{-6} (0.539)	99.67±1.36	100.07±1.8
10^{-7}(0.0539)	100.38±0.99	100.28±0.98

[a] *Average of three determinations*

CONCLUSION

The described sensors are sufficiently simple and selective for the quantitative determination of STG in pure form, pharmaceutical formulations, in its combined dosage form, in plasma and urine. The proposed sensors offer advantages of fast response and elimination of drug pre-treatment or separation steps. They can therefore be used for routine analysis of STG in quality-control laboratories.

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