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MIP-Based Biomimetic Sensors for Static and Hydrodynamic Potentiometric Transduction of Sitagliptin in Biological Fluids

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Solid contact potentiometric membrane sensors for sitagliptin (STG) incorporated with molecular imprinted polymer (MIP) were synthesized and implemented. The sensors were fabricated with conventional and tubular configurations with a graphite-based electrical contact, and no internal reference solution. The selective membranes consist of sitagliptin-methacrylic (MIP/MAA) or 2-vinyl pyridine (MIP/2-VP)-ethylene glycol methacrylate (EGDMA) electroactive materials dispersed in a PVC matrix of *o*-nitrophenyloctyl ether (*o*-NPOE) plasticizer. The determination of STG was carried out in acidic solution at pH 5, where positively charged species predominated prevalently. The suggested sensors exhibited marked selectivity, sensitivity, long term stability and reproducibility. At their optimum conditions, the sensors displayed wide concentration ranges of $5.0x10^{-6}$ – $1.0x10^{-2}$ mol L^{-1} and $1.0x10^{-5}$ – $1.0x10^{-2}$ mol L^{-1} with slopes of about 52.7– 40.5 mV $decade^{-1}$; respectively. Sensors exhibit detection limits of $2.6x10^{-6}$ and $5.3x10^{-6}$ mol L^{-1} upon the use of MAA and 2-VP monomers in the imprinted polymer, respectively. Validation of the assay method according to the quality assurance standards (range, within-day repeatability, between-day variability, standard deviation, accuracy, and good performance characteristics) which could assure good reliable novel sensors for STG estimation was justified. Application of the proposed flow-through assay method for routine determination of STG in pharmaceutical formulations and biological fluids carried out.

Keywords: Molecularly imprinted polymers; Potentiometric sensors; Sitagliptin; Flow injection analysis (FIA); Biological fluids.

1. INTRODUCTION

Type-2 diabetes mellitus is a long-term metabolic disorder wherein the body becomes resistant to the effects of insulin, a hormone that regulates sugar absorption, though it still normally secreted by the patient pancrease. According to the American Diabetes Association, this disease affects up to 45%

of individuals above 65 year-old, and involves at least 90% of diabetes patients above 20 year-old [1]. The most preferred option to treat this disease is to decrease glucose levels in blood by administration of antidiabetic drugs. Currently, metformin is the most prescribed anti-diabetic drug in the world and constitutes the primary first line therapy for treatment of type II diabetes.

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), marketed as Januvia™ by Merck and Sharp and Dohme, is a relatively new oral anti-hyperglycemic drug used to treat type II diabetes. STG competitively inhibits dipeptydyl peptidase IV (DPPIV), an enzyme involved in the breakdown of incretins such as glucagon-like particle-1 (GLP-1) which potentiate insulin secretion in vivo. Inhibition of DPPIV reduces the breakdown of GLP-1 and increases insulin secretion; this suppresses the release of glucagon from the pancreas and drives down blood sugar levels [2]. Merck and Co. also market STG in combination with metformin in a single dosage form as Junumet™.

Analytical methods for the analysis of STG in biological samples are required for therapeutic drug monitoring and the complete understanding of pharmacokinetic mechanisms such as absorption, distribution, metabolism and elimination. In addition, appropriate dose adjustments of STG are needed for patients with impaired renal function [3]. Therefore, analysis of STG in urine samples has critical importance. In the literature, there are tandem mass spectrometry method, liquid chromatography methods and gas chromatography-mass spectrometry method for the determination of STG alone and in combination with metformin in biological samples [4-14]. However, the chromatographic studies have problems such as low capacity factor, unshaped or non-splitted peaks [4-9]. Analytical methods are also reported for the determination of STG by spectrophotometry [15-18], spectofluorometry [19,20], electrophoresis [21] and potentiometry [22] have been used. However, one of the major drawbacks associated with some of these methods is their low selectivity. Therefore, it is important to develop techniques for the rapid and selective extraction of STG from biological matrices.

Alternative and advantageous methods should rely on expeditious and efficient procedures providing highly specific and sensitive measurements. Ion-selective sensor's utility and simplicity have replaced for long other wet analytical methods, because they offer high precision and rapidity, low cost of analysis, enhanced selectivity and sensitivity over a wide range of concentrations [23,24]. Improved selectivity may also be achieved by means of using MIP sensing elements [25].

Molecularly imprinted polymers (MIPs) possess pre-defined specific cavities designed for target molecules. These are stable to extremes of pH, organic solvents and temperature which provides for more flexibility in the development of analytical and bioanalytical methods [26-32].

Application of MIP sensors to potentiometry may provide some advantages. The creation of a membrane potential does not require the template to be extracted from the membrane [33] reducing a possible source uncertainty at the determination or a sensitivity limiting factor. There are also no size restrictions on the template compound because species do not have to diffuse through the membrane [33].

In the present work, molecularly imprinted polymer (MIP) membranes for STG were prepared. The STG imprinted polymers used were prepared from 2-vinyl pyridine (2-VP) or methacrylic acid (MAA) monomers in the presence of ethylene glycol dimethylacrylic acid (EGDMAA), which avoids a higher-level of cross-linking network. The polymers could be regarded as an artificial receptor to

recognize STG by a stereo-shape ability, stoichiometric non-covalent interactions as well as induced polarization between MIP and STG. Polymeric membrane sensors incorporating the MIP, were prepared, characterized, compared and used for determination of STG in real samples. Potentiometric sensors based on the prepared MIP offer the advantages of lower detection limit, fast response time, long term stability, near-Nernstian slope over a wide range of concentration, and good selectivity for STG over most common cations and analog drugs. The sensors were also evaluated in a flowing media, and applied to the analysis of complex samples.

2. EXPERIMENTAL

2.1. Apparatus

All potential measurements were made at 25±1 ^{o}C with an Orion (Cambridge, MA, USA) Model 720 SA pH/mV meter using STG membrane sensor in conjunction with an Orion Ag/AgCl double-junction reference electrode (Model 90-20) filled with 10% (w/v) KNO_3. A combination Orion Ross glass electrode (81-02) was used for pH adjustments.

Flow injection analysis (FIA) manifold consisted of a two-channel Ismatech Ms-REGLO model peristaltic pump, polyethylene tubing (0.71 mmi.d.) and an Omnifit injection valve (Omnifit, Cambridge, UK) with a sample loop of 100 µL volume. The potential signals were recorded using an Orion (Cambridge, MA, USA) Model 720A pH/mV meter connected to a PC through the interface ADC 16 (Pico Tech, UK) and Pico Log for windows (version 5.07) software.

2.2. Chemicals and materials

Sitagliptin MK-0726, 100%, was obtained from Merck Sharp and Dohme Co. (USA). Acetaminophen, caffeine, pheniramine, dextromethorphan, nicotine, pseudoephedrine, diphenhydramine and acetylsalicylic acid were purchased from Acros Organics (B.V.B.A., Belgium). Ethylene glycol dimethacrylate (EGDMA), 2-vinyl pyridine (2-VP) and methacrylic acid (MAA), high molecular weight poly (vinyl chloride) PVC, potassium tetrakis (p-chlorotetraphenylborate) (pClTPB), tridodecylmethylammonium chloride (TDMAC) and *o,* nitrophenyl octyl ether (*o,*NPOE) were used as received from Fluka (Ronkonoma, NY). Tetrahydrofuran (THF) was obtained from Riedel-deHaen. Benzoylperoxide (BPO) was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). The buffer used in this work was 0.01 mol L^{-1} acetate buffer at pH 5.

2.3. Polymer synthesis

Molecularly imprinted polymers with STG were prepared by using methacrylic acid (MAA, 4 mmol) or 2-VP (4-mmol) as functional monomers, ethylene glycol dimethacrylate (EGDMA, 20 mmol) as a cross-linker and acetonitril (3 mL) as the porogen. The template-monomer mixture and solvent were transferred to a test tube and benzoylperoxide (BPO, 80 mg) as an initiator was added. The mixture was degased by bubbling N_2 for 5 min. The tube was sealed and heated in a water bath at

80 ^{o}C for 1 h. The control blank polymers (NIPs) were prepared using an identical procedure but in absence of the template. After polymerization, the polymers were grinded and sieved to particle sizes ranging 50 and 150 mm. Finally the particles were washed with methanol: acetic acid (1: 1) and with acetonitrile : acetic acid (1 : 1) to remove interfering compounds arising from the synthesis (templates and unreacted monomers). All polymers (MIP/MAA, NIP/MAA, MIP/2-VP and NIP/2-VP) were let dry at ambient temperature, before their use as potentiometric sensors.

2.4. Sensor construction

The STG-selective membranes for solid-contact ISEs contained MIP/MAA [ISE I] or MIP/2-VP [ISE II] (2.7 wt%, 15 mg), *o*-NPOE (63.6 wt%, 350 mg), and PVC (33.6 wt%, 185 mg). The membranes were prepared by dissolving the components (in total, 550 mg) in THF (3 mL). The membrane solutions were cast into a conductive supports of conventional or tubular shapes and left overnight for evaporating and yielding transparent membranes. The lifetime of the membranes was 1 month. The sensors were conditioned by soaking in $1.0x10^{-3}$ mol L^{-1} STG solution for 2 hours and stored dry when not in use. The sensors were calibrated by transferring 1.0 mL aliquots of $1.0x10^{-6}$–$1.0x10^{-2}$ mol L^{-1} to a beaker containing 10 mL of $1.0x10^{-2}$ mol L^{-1} acetate buffer of pH 5.0. The sensors were immersed in the solution in conjunction with a double junction Ag/AgCl reference electrode. The potential readings of the sensors were recorded after stabilization to ±0.2 mV and the emf was plotted as a function of logarithm STG concentration. The calibration graph was used for subsequent determination of unknown STG concentrations.

2.5. Binding experiment

The polymer particles (MIP/MAA, MIP/AA and MIP/MAA-AA) were repeatedly washed with ethanol/acetic acid (5:1, v/v) until spectrophotometric measurements of the supernatant showed no peak of STG at 275 nm. After being thoroughly washed with ethanol and dried, the polymer (20 mg) was immersed in a various concentrations of STG ranging from 0.2–2 mmol L^{-1} at 25 ^{o}C. After the incubation for 20 h, the sample tubes were centrifuged. Aliquots of the supernatant were taken and analyzed by spectrophotometry to quantify the concentration of free sitagliptin, *F*, and subsequently the amount of STG bound to the polymer, *B*. Three independent batches were tested for each concentration. The average data were used for subsequent analysis. For Scatchard analysis, *B/F* is plotted versus *B* according to the equation, $B/F = B_{max} - B/K_d$, where K_d is the equilibrium dissociation constant.

2.6. Determination of sitagliptin in pharmaceutical formulations

Three intact Januvia® tablets (nominally containing 100.0 mg of sitagliptin phosphate monohydrate per tablet, Merck Sharp and Dohme Co., Pavia, Italy) and Janumet® tablets (nominally containing a combination of 50.0 mg of sitagliptin phosphate monohydrate and 1000 mg of metformin

per tablet, Merck Sharp and Dohme Co., Cairo, Egypt) were accurately weighed and the average weight of the whole tablets was determined. The tablets were emptied and the average content of one tablet was determined. Januvia® and Janumet® suspension (5 mg mL^{-1}) were directly used after appropriate dilution. The test solutions were sonicated at room temperature for 10 min to ensure complete drug dissolution. The test STG solutions were potentiometrically determined using the proposed sensor and both direct potentiometry and standard addition (spiking) methods [34]. For continuous measurements (FIA), a 100 μL aliquot of the drug test solution was injected in triplicate as described above and the average potential reading was compared with the calibration plot.

2.7. Determination of sitagliptin in spiked urine and serum samples

Aliquots of biological fluids (1.0 mL of human serum or 5.0 mL of human urine) were transferred to a 10 mL volumetric flask. Aliquots (1.0 - 4.0 mL) of standard $5.0x10^{-4}$ mol L^{-1} STG were added and the solution was completed to the mark with $1.0x10^{-2}$ mol L^{-1} acetate buffer of pH 5.0. The solutions were thoroughly mixed and the drug concentration was measured as described previously. The sensor was stored in acetate buffer of pH 5.0 between measurements and can be used for up to 2 weeks.

3. RESULTS AND DISCUSSION

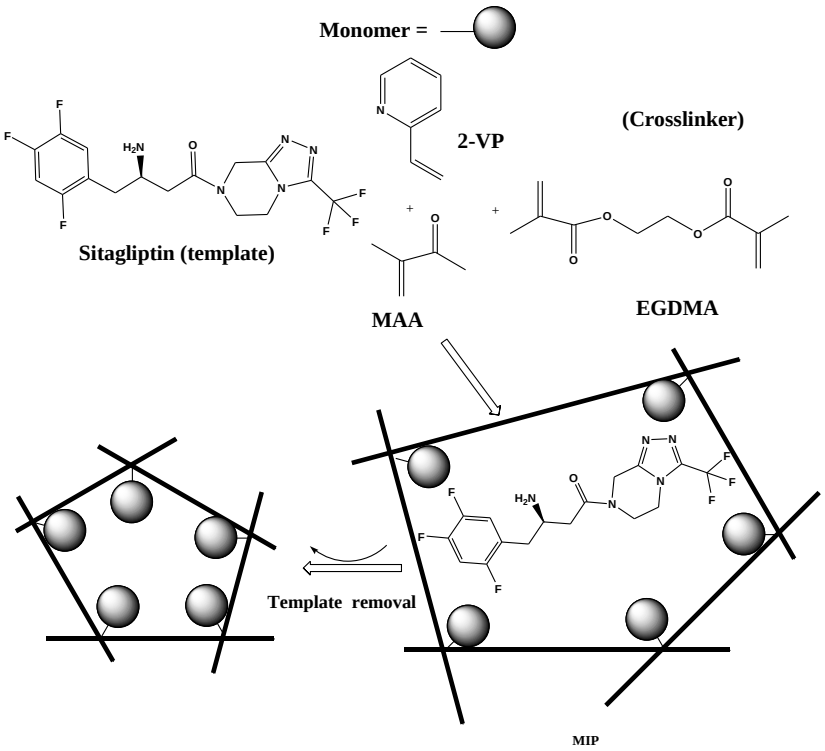


Figure 1. A schematic illustration for the molecular imprinting process.

A strong binding between an ionophore and its target ion may enhance the selectivity and the sensitivity of an ISE [35]. The design of highly specific imprinted must be made avoiding the covalent binding of the template molecules to the tailored-cavities, since it would compromise the fast equilibrium and reversible binding by reusable potentiometric sensing devices [36].

Therefore, we aimed to establish a simple and sensitive analytical system based on MIPs. For this purpose, we proposed an electrochemical sensor utilizing the potentiometric determination method of bound analyte to the MIPs by electrochemical reaction. A schematic illustration for the molecular imprinting process is shown in Fig.1.

3.1. Scatchard analysis

To testify the influence of interaction strength between the template and functional monomers on imprinting effect, the MIPs using MAA and 2-VP as the functional monomers were synthesized. All polymers were analyzed for binding template using equilibrium binding experiments [36]. It can be seen that among the MIPs prepared using the two different types of functional monomers, only MIP/MAA shows higher binding affinity than MIP/2-VP for the template. This can be attributed to the absence of significant strong interaction between 2-VP functional monomer and the template molecules. In view of the structure of these functional monomers, MAA is a kind of acidic functional monomer which serves as not only a proton donor, but also a proton acceptor. Its carboxyl group may form a typical three-point interaction with the amine groups and the fluoro groups of STG by hydrogen bonding and/or ionic bonding.

In addition, the basic nitrogen in 2-VP is only likely to involve hydrogen bonding with the amino group of STG. Thus, a stable host—guest complex between template and functional monomer is formed in the imprinting process. The existence of such a complex leads to the formation of well-defined specific binding sites in imprinted polymers. The equilibrium dissociation constant K_{d1} and the apparent maximum amount B_{max1} for the higher affinity binding sites can be calculated to be 2.8 and 1.3 mmol L^{-1} and 13.7 and 11.8 mmol g^{-1} for MIP/MAA and MIP/2-VP, respectively. By the same treatment, K_{d2} and B_{max2} for the lower affinity binding sites were calculated to be 5.5 and 6.7 mmol L^{-1} and 15.3 and 13.6 mmol g^{-1} for MIP/MAA and MIP/2-VP, respectively. Consequently, MAA-imprinted polymer showed a higher molecular imprinting effect compared with 2-VP.

3.2. Sensor performance characteristics

The synthesized MIP's were incorporated into the PVC membrane and were tested as sensing materials in the proposed potentiometric sensor. The potential response obtained with the sensors prepared with STG-MIP membrane are given in Fig. 2. As seen from the figure, the sensors exhibit linear potentiometric response to STG ions with lower limit of linear range 5.0×10^{-6} and 1.0×10^{-5} mol L^{-1}, and detection limits of $2.6\text{x}10^{-6}$ and $5.3\text{x}10^{-6}$ mol L^{-1}, for sensors based on MAA and 2-VP polymers, respectively. All sensors exhibit near-Nernstian slopes of 52.7±1.5 (r^2 =0.9994) and

40.1±1.1 (r^2 =0.9997) mV decade^{-1}, respectively. The sensors NIP's did not exhibit linear response in all range of work concentration.

A comparison between the membranes without ionic additive and that containing anionic additive (i.e. 30 mol % TPB^- relative to the ionophore) showed that incorporation of TPB^- in STG sensors caused a remarkable enhancement of the potentiomtric response properties. The slope increased to 64.1±0.3 and 51.8±0.9 mV decade^{-1}, linear dynamic range extended from5.0 x 10^{-6} and 8.0x10^{-6} to 1.0 x 10^{-2} mol L^{-1}, and the detection limit decreased to 2.0x10^{-6} – 2.5x10^{-6} mol L^{-1}. The incorporation of cationic site additive (i.e. 30 mol % $TDMA^+$ relative to the ionophore) dramatically deteriorated the potentiometric response characteristics showing a slope of 23.0 ± 1.2 mV decade $^{-1}$, detection limit of 3.0 x 10^{-3} mol L^{-1} and linear response range begins from 5.0 x 10^{-3} mol L^{-1}. The composition and potentiometric response characteristics of the membrane sensors incorporating the prepared MIP as recognition elements alone and in the presence of TPB^- as anionic site are shown in Table 1.

Table 1. Potentiometric response characteristics of sitagliptin membrane based sensors

Parameter	MIP/MAA	MIP/MAA+TPB^-	MIP/2-VP	MIP/2-VP+TPB^-
Slope (mVdecade^{-1})	52.7	64.1	40.5	51.8
Correlation coefficient, r (n=5)	0.9994	0.9995	0.9997	0.9999
Linear range (mol L^{-1})	5.0x10^{-6} 1.0x10^{-2}	5.0x10^{-6} 1.0x10^{-2}	1.0x10^{-5} 1.0x10^{-2}	2.5x10^{-6} 1.0x10^{-2}
Detection limit (mol L^{-1})	2.6x10^{-6}	2.0x10^{-6}	5.3x10^{-6}	8.0x10^{-6}
Working range (pH)	4.4 - 6.5	4.4 - 6.5	4.4 - 6.5	4.4 - 6.5
Response time (s)	< 20	< 20	< 5	< 5
Standard deviation σ_v (mV)	2.2	2.3	1.1	0.6
Repeatability, Cv_w (%)	1.1	0.9	0.8	1.4
Accuracy (%)	99.4	99.6	99.7	99.2

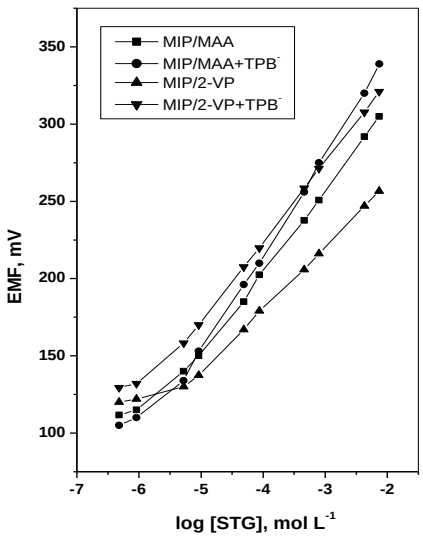


Figure 2. Potentiometric response of Sitagliptin PVC membrane sensors under static mode of operation.

Replicate measurements (n=10) of an internal quality control (IQS) sample (10.0 μg mL^{-1} of certified reference STG) gave an average results of 9.8±0.3 μg mL^{-1}. Calculation of the student's (*t*) value at 95% confidence level was made using Equation 2:

$$t_{exp} = (\mu - \chi)\ x\ n^{1/2}/S \qquad (2)$$

Where: μ is the concentration of the initial internal quality control sample, χ is the average concentration found, *n* is the number of replicates analyzed and *S* is the standard deviation of measurements. No statistical difference was detected between the practically obtained (t_{exp}=1.55) and the theoretically tabulated (t_{tab}=1.833) values. Thus the null hypothesis is retained and the method accuracy is acceptable.

The stability of sensors were monitored continuously at 1.0×10^{-4} mol L^{-1} of STG solution and evaluated for a period of 5 h, the potential drift obtained was ≤ 1.1 mV h^{-1}. The repeatability of the potential reading for the sensors was examined by subsequent measurements in 5.0×10^{-4} mol L^{-1} of STG solution immediately after measuring the first set of solution at 1.0×10^{-4} mol L^{-1} of STG solution. The response properties of the proposed electrode did not change obviously after the use of the electrode for three months.

The robustness of the method was evaluated by testing the influence of pH variation and measuring time on the accuracy of the results. The effect of pH on the potentiometric response of MIP/MAA and MIP/2-VP based membrane sensors was examined with standard $1.0x10^{-4}$ and $1.0x10^{-3}$ mol L^{-1} STG solutions over a pH range of 2–11. The pH of the solution was adjusted with either hydrochloric acid and/or sodium hydroxide solutions. The results indicate that the variation of solution pH over the range 4.4–6.5 has no significant effect on the sensor response. Since the pKa of STG is 7.7 [37], 2 and 3 pH units below the pKa resulted in 99 and 99.9 % ionization (protonation) of the drug, respectively. The potential of the sensor considerably declined with negative drift at higher pH values probably due to progressive precipitation of the free STG base. At pH<3, the sensor response was severely influenced by H_3O^+.

The effect of measurement time on the accuracy of the results was also tested. Stable potential readings were obtained within 10–20 seconds for MIP/MAA and < 5 seconds for MIP/2-VP for $1.0x10^{-2}$–$1.0x10^{-6}$ mol L^{-1} STG test solutions and the accuracy of the results did not significantly affected by increasing the measurement time up to 10 min.

The ruggedness of the potentiometric method was also evaluated by carrying out the analysis using six different electrodes and two different instruments on different days.

A relative standard deviation (RSD) of less than 1.0 was observed for repetitive measurements during three different days (n=10). The results indicate that this method is capable of producing results with high precision and stability.

It is clear that the present suggested method has several advantages. These are the low cost, fast response, minimum manipulation steps, and wide range of linear response, lower detection limit, high accuracy, good precision, applicability to turbid and colored solutions and possibility to interface with automated systems.

3.3. Sensors selectivity

Table 2. Selectivity coefficient values for sitagliptin selective sensors as calculated by fixed interference method

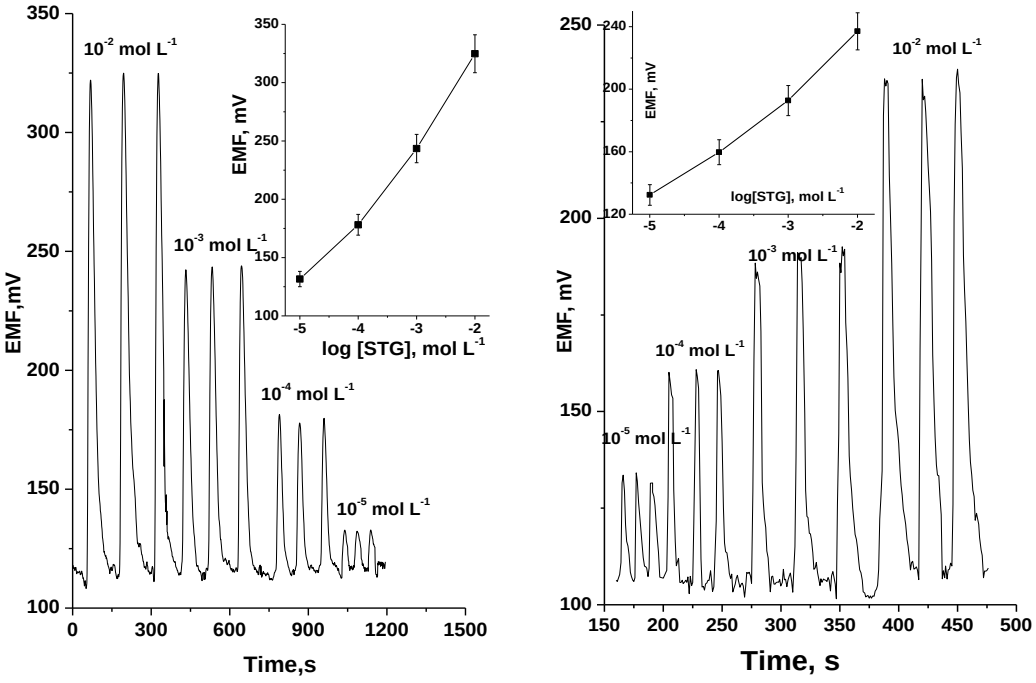
Interferent	$Log\ K^{pot}_{SIT,\ J}$			
	MIP/MAA	MIP/MAA+TPB$^-$	MIP/2-VP	MIP/2-VP+TPB$^-$
Sitagliptin	0	0	0	0
Quinine	-1.5	-1.4	-1.6	-1.6
Caffeine	-2.7	-2.5	-2.4	-2.3
Pseudoephedrine	-1.8	-1.7	-1.8	-1.8
Histidine	-2.4	-2.5	-2.3	-2.2
Glutamine	-2.6	-2.5	-2.2	-2.1
Fluoxetine	-2.8	-2.7	-2.9	-2.9
Pheniramine	-1.9	-1.8	-2.0	-2.0
Dextromethorphan	-1.7	-1.6	-1.5	-1.4
Diphenhydramine	-3.0	-2.9	-2.9	-2.8
Metformin	-2.1	-2.0	-1.8	-1.7
Nicotine	-1.7	-1.7	-1.6	-1.5
Creatine	-2.7	-2.7	-2.6	-2.5
Creatinine	-2.9	-2.8	-2.8	-2.8
Na^+	-4.1	-4.2	-4.2	-3.9
K^+	-2.1	-1.8	-2.5	-2.5

The performance of the sensors in the presence of some cations was assessed by measuring the selectivity coefficient values $Log\ K^{pot}_{STG,J}$ using the fixed interference solutions method [38]. Potentiometric selectivities of the sensors are related to the preferential interaction of the mimicked receptors with STG in 0.01 mol L^{-1} acetate solution of pH 5. Table 2 presents potentiometric selectivities of the proposed STG sensors over various related compounds, basic drugs, inorganic cations, and additives commonly used in the drug formulations (e.g. K^+, Na^+, fluoxetine, caffeine, pheniramine, dextromethorphan, nicotine, pseudoephedrine, diphenhydramine, metformine, creatine, glutamine, creatinine, histidine and quinine). Glucose, maltose, starch, talc, and tween-80 used as drug excipients at concentration level as high as 1000-fold excess over STG have no diverse effect on the accuracy of the results.

3.4. Optimization of Parameters in FIA Mode

The dependency of the peak heights and peak width (and time to recover the baseline) with flow rate was studied using the sensor response to 1.0×10^{-4} mol L^{-1} solution of sitagliptin. As the flow rate increased, the peaks became narrower and increased in height to a near plateau at a flow rate of 3.0 mL min^{-1}. However, at higher flow rates, the peak height started to decrease slightly. Thus, a flow rate of 4.0 mL min^{-1} was selected as an optimum value for further studies. With a flow rates less than 3.0

mL min^{-1}, the sensor showed slight memory effect and required long washing time, to recover the base line leading to a decrease in the number of sample outputs. Variation or fluctuation of the base line did not exceed ±5% of the peak height. The repeatability of the sensor response was excellent; peak height relative standard deviation [R.S.D. (%)] for 5 injections of 1.0×10^{-4} and 5.0×10^{-4} mol L^{-1} solutions were 0.9 and 1.2%, respectively. A linear relationship between STG concentrations and FIA signals was obtained over the range 5.0×10^{-5}–1.0×10^{-2} mol L^{-1} and 2.0 $x10^{-4}$ – $1.0x10^{-2}$ with a slope of 73.4±0.6 and 37.8±2.1 mV $decade^{-1}$ and lower detection limit of 1.0×10^{-5} and $8.0x10^{-5}$ mol L^{-1} at a signal/noise (S/N) ratio of ±1.5 for MIP/MAA and MIP/2-VP membrane based sensors, respectively (Fig. 3).



(A) (B)

Figure 3. Typical (FIA) peaks produced by injection of 100 µL aqueous solutions of standard STG into a stream of 10^{-2} mol L^{-1} acetate buffer pH 5 flowing at 4.0 mL min^{-1} using: (A) MIP/MAA and (B) MIP/2-VP membrane based sensors.

3.5. Determination of sitagliptin in pharmaceutical formulations

The potentiometric STG membrane sensors can be used for routine analysis and quality control/quality assurance during manufacture of sitagliptin phosphate. The use of the sensor in a FIA mode of operation shorten the assay time, allow the use of little sample quantities for drug detection in both parent and related pharmaceutical preparations. Potentiometric determination of sitagliptin in drug formulations under static mode of operation was carried out using both direct potentiometry and the standard addition (spiking technique). With the direct potentiometic technique, the recoveries were

98.3±1.1and 97.2±0.9%. The standard addition method showed results with recoveries of 97.8±1.4 and 98.1±0.7% (Table 3).

Table 3. Determination of sitagliptin phosphate in some pharmaceutical preparations using MIP/MAA based membrane sensor.

Sample	Labeled, mg tablet^{-1}	Recovery found * (%)			
		Direct potentiometry	Standard addition	FIA	Spectrophotometry [18]
Januvia® tablets (Merck Sharp and Dohme Co., Pavia, Italy)	100	98.3±1.1	97.8±1.4	96.7±0.8	98.4±1.3
Janumet® tablets (Merck Sharp and Dohme Co., Cairo, Egypt)	100	97.2±0.9	98.1±0.7	97.2±1.3	98.7±1.4

*Average of 5 determinations

With the flow injection technique, the recoveries were 96.7±0.8% and 97.2±1.3%. These data were compared with results obtained by spectrophotometric method [18]. An *F* test showed no significant difference at 95% confidence limit between the means and variances of the results. The calculated *F* values (n=10) of the results obtained by the present sensor and different potentiometric techniques (Table 3) for drug tablets were less than 2.19, compared with the theoretical tabulated value (*F*=3.18).

3.6. Sitagliptin assay in biological fluids

Table 4. Potentiometric determination of sitagliptin phosphate in spiked human urine and plasma samples using MIP/MAA based membrane sensor.

Matrix	Spiked concentration (µg mL^{-1})	Recovery found* (%)	
		Batch	FIA
Urine	20.0	95.2±1.4	94.4±1.3
	25.0	96.7±0.9	98.1±1.1
	30.0	95.8±0.5	96.9±1.7
	40.0	98.2±0.4	98.1±0.3
Serum	20.0	95.1±0.8	94.4±2.1
	25.0	96.2±1.2	95.3±0.3
	30.0	98.6±0.4	97.4±0.3
	40.0	99.1±0.3	98.4±0.5

Application of the method for determining STG in biological fluids was tested by spiking aliquots of serum or urine samples with a known standard of STG. The results show average recoveries (accuracy) of 96.5±0.7% and 96.9±1.1% and 97.3±0.7% and 96.4±0.8% in urine and serum samples using batch and FIA techniques, respectively (Table 4). This confirms the applicability of the method for accurate routine analysis of STG in biological fluids. The sensors can be used for up to 4 weeks before noticeable drift is detected, probably due to contamination of the PVC membrane with the serum or urine matrix.

4. CONCLUSIONS

A STG potentiometric sensors were fabricated from an MIP based on the use of MAA or 2-VP as a functional monomer exhibited excellent potentiometric performances such as fast response, a wide working pH range, high sensitivity, long-term stability, good selectivity and automatic feasibility. The use of these sensors as detectors for the continuous monitoring of STG^+ offered an advantage of simple design, ease of construction and possible application in the routine control of STG^+ ions samples. Optimization and full validation of the assay method enable accurate, precise and rapid measurements of as low as $2.6x10^{-6}$ mol L^{-1} and $5.3x10^{-6}$ mol L^{-1} STG^+ ions in different samples for (MIP/MAA) and (MIP/2-VP) membrane based sensors, respectively. No pretreatment or prior separation steps are used.

Application to STG evaluation in the routine control of pharmaceutical drug solutions and biological fluids revealed good results. The results are favorably compared with data obtained using the standard method [18].

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