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Highly Sensitive Potentiometric Assay of Vardenafil in Pharmaceutical Formulations and Biological Fluids

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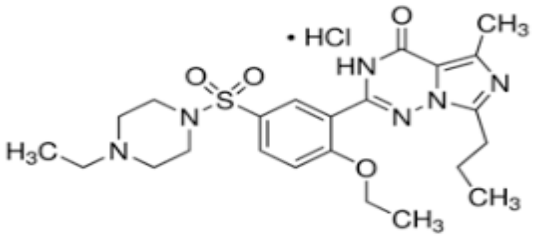
Abstract

Sensitive disposable screen printed potentiometric vardenafil (VAR) sensors have been constructed based on multiwall carbon nanotubes/crown ether (MWCNTs/CE) composite. Factors affecting the sensor performance have been optimized on the basis of nature and content of the molecular recognition elements, anionic sites, membrane plasticizers and nanomaterial. The fabricated sensors showed acceptable selectivity and sensitivity within the vardenafil concentration range from 10^{-6} to 10^{-2} mol L^{-1} with Nernstian slope value of 62.1±0.8 mV $decade^{-1}$ and detection limit 8.0 × 10^{-7} mol L^{-1}. Fast response time, enhanced potential reading stability and long shelf lifetime are the promising futures of the novel sensors. The proposed method has been suggested for potentiometric assay of VAR in dosage formulation and biological fluids with average recoveries agreeable with the reported official methods.

Keywords: Vardenafil hydrochloride; Disposable screen printed sensors; Crown ethers; Carbon nanotubes; Pharmaceutical and biological samples

1. Introduction

Vardenafil (VAR), 2-{2-ethoxy-5-[(4-ethylpiperazin-1-yl)sulfonyl]phenyl}-5-methyl-7-propyl -1H,4H-imidazo[4,3-f][1,2,4]triazin-4-one, is a benzene sulfonamide derivative (**Scheme 1**). Vardenafil selectively inhibits the phosphodiesterase 5 (PDE5), thus inhibiting cyclic guanosine monophosphate degradation (cGMP) found in the smooth muscle of the penis corpus cavernosa and corpus spongiosum [1]. cGMP degradation inhibition results in extended muscle relaxation, vasodilatation and corpus cavernosa blood engorgement, thus prolonging penile erection. In the corpus cavernosum, nitric oxide is released during sexual stimulation from the nerve endings and endothelial cells which activates the guanylate cyclase enzyme, resulting in an increased smooth synthesis of cyclic guanosine monophosphate (cGMP) in the smooth muscle cells of the corpus cavernosum.



Scheme 1: Chemical structure of vardenafil hydrochloride

In turn, the cGMP stimulates smooth muscle relaxation, enabling increased blood flow to the penis, leading to erection. The tissue concentration of

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cGMP is controlled by both synthesis rates and phosphodiesterase (PDE) degradation rates.

Chromatographic separation techniques are the official pharmacopeial methods for VAR determination in pharmaceutical formulations [2]. Spectrophotometric and chromatographic tools are the most common techniques found in research literature. Abdel-Moety et al., [3] reported a simple, accurate and sensitive UV-spectrophotometric method for simultaneous determination of vardenafil (VAR) and dapoxetine (DAP) in bulk powder and combined dosage form. Following, charge transfer complexation reactions with various π-acceptors: 7,7,8,8-tetracyanoquinodimethane (TCNQ), 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) and chloranilic acid (p-CLA) were applied for the spectrophotometric determination of VAR in pharmaceutical preparation [4]. Beer's law is obeyed in the concentration range from 5.0 to 200 mg mL^{-1} with good correlation coefficient. More sensitive spectrophotometric protocol based on oxidative coupling of vardenafil with 3-methyl-2-benzothiazolinone hydrazine hydrochloride in presence of ferric chloride in acidic medium yielding green colored chromogen with absorption maxima at 625 nm or oxidation of 4-aminoantipyrine by potassium periodate, which subsequently couples with vardenafil in an alkaline medium to form a red colored product having absorption maxima at 530 nm was found in literature [5].

High performance liquid chromatographic methods with different detectors including HPLC-UV [6,7], HPLC-fluorescence [8] HPLC-chemiluminescence [9], HPLC-amperometric detection [10], HPLC-diode array and electrospray-mass spectrometry [10] have been reported. Gas chromatography/mass spectrometry [11] and LC-MS/MS [12] had been applied for determination of VAR and its metabolites in biological fluids.

However, the aforementioned techniques require sophisticated and expensive equipments in addition to application of toxic organic solvents which are not affordable for smaller laboratories. Contrary, electroanalytical techniques are now well-established technique for pharmaceutical analysis having the advantage of instrumentation simplicity, acceptable precision, and accuracy [13-17].

An adsorptive anodic stripping voltammetry at a carbon paste electrode [18] or at pencil graphite disposable electrodes [19] were reported for trace determination of vardenafil hydrochloride in commercial formulation and human serum. For more simple instrumentation and equipment, potentiometric protocols can be introduced for determination of VAR. In this regard, simple and accurate potentiometric titration of vardenafil and dapoxetine hydrochlorides against N-bromosuccinimide (NBS) was developed using a platinum indicator electrode [20]. The method was successfully applied for the determination of two drugs in their pure state or pharmaceutical preparations with mean recovery values of 99.76-99.73% and relative standard deviations (RSD) were 0.46-0.62% for vardenafil and dapoxetine hydrochlorides, respectively. To the best of our knowledge, only a PVC membrane VAR sensor based on VAR- tetraphenyl borate as electroactive material was found in the literature [21]. The cited electrodes showed Nernstian slope value of 54.42±0.38 mV $decade^{-1}$ in the VAR concentration range 10^{-2}-10^{-5} mol L^{-1}. PVC membrane electrodes are mechanically complicated with short operational lifetimes due to leaching of the electroactive material into the internal reference and the bathing solution. In addition, such electrodes are usually inconvenient for biomedical analysis due to the difficulty of miniaturization and necessity for sterilization; therefore, disposable sensors are more favorable. More recently, production of disposable planer electrochemical sensors with large-scale and prolonged shelf-lifetime applying screen printing technology was reported [22-26]. This methodology supports sensor miniaturization with portable devices and establishes its route from “lab-to-market” for a plethora of sensors.

The aim of the present work was to incorporate crown ether (CE) as molecular recognition element and carbon nanotubes to the electrode matrix for fabrication of disposable screen printed sensors for potentiometric determination of vardenafil in dosage forms and biological samples.

2. Experimental

2.1 Reagents and chemicals

All reagents were of the analytical grade and bidistilled water was used throughout the experiments. Cyclodextrins including native *α*-(I, *Sigma*), *β*-cyclodextrins (II, *Sigma*), *γ*-(II, *Sigma*) and methylated derivatives heptakis (*2,6-di-O-methyl)-β-*

cyclodextrin (IV, *Aldrich*), heptakis (*2,3,6-tri-O-methyl)-β*-cyclodextrin (V, Aldrich) were tested as sensing elements. Other macromolecules including crown ethers and calixarenes including; 12-crown-4 ether (VI, *Fluka*), 15-crown-5 ether (VII, *Fluka*),18-crown-6 ether (VIII, *Fluka*), 21-crown-7 ether (IX, *Fluka*), dibenzo 24-crown-8 ether (X, *Fluka*), 30-crown- 10 ether (XI, *Fluka*), calix[4]arene (XII, *Aldrich*) or calix[8] arene (XIII, *Aldrich*) were also incorporated in the electrode matrix.

Sodium tetraphenylborate (NaTPB, *Fluka*), sodium tetrakis (4-fluorophenyl) borate (NaTFPB, *Sigma*) and potassium tetrakis (4-chlorophenyl) borate (KTClPB, *Fluka*) were applied as anionic additives. Membrane plasticizers with different dielectric constants including; *o*-nitrophenyloctylether (*o*-NPOE, *Sigma*), 2-fluorophenyl 2-nitrophenyl ether (*f*-PNPE, *Fluka*), dioctylphthalate (DOP, *Sigma*) and tricresylphosphate (TCP, *Fluka*) were used as membrane plasticizers. Poly (vinyl chloride) (PVC, relative high molecular weight, *Aldrich*), graphite powder (synthetic 1– 2 µm, *Aldrich*), silver and silver chloride powders (*Sigma*) were used for fabrication of the printing ink. Different carbonaceous nanomaterials single-wall carbon nanotubes (SWCNTs, *Aldrich*), multi-wall carbon nanotubes (MWCNTs, *Aldrich*), and graphene nanosheet (Gr, *Sigma*) were used.

Solutions of the tested interferents including: Li^+, NH_4^+, Ca^{+2}, Mg^{+2}, Ni^{+2}, Co^{+2}, phosphate, citrate, maltose, starch, sucrose, microcrystalline cellulose, crospovidone, croscarmellose sodium, glucose, fructose, glycine, caffeine and cysteine were prepared from appropriate amounts of the corresponding analytical grade chemicals and used in selectivity measurements.

2.2. Authentic sample

Vardenafil hydrochloride authentic sample ($C_{23}H_{33}ClN_6O_4S$, 525.1 g mol^{-1}) was supplied by the Standard Department, National Organization for Drug Control and Research (NODCAR), Giza, Egypt). The stock drug solution was freshly prepared by dissolving the appropriate amounts of VAR in bidistilled water. Working VAR solutions covering the concentration range from 1×10^{-2} to 1×10^{-7} mol L^{-1} were prepared by further appropriate dilution of the stock solution with bidistilled water.

2.3. Pharmaceutical preparations

Different pharmaceutical formulations (Powerecta 20 mg, EVA Pharma; Rectivard 20 mg Marcyrl Co; Vardapex 10 mg, Multi Apex; Romantigra 20 mg, Global Napi, Cairo, Egypt) were collected from local drug stores. Two tablets were weighed, grinded and an accurate weight of the powder assigned to contain 20 mg VAR were dissolved in bidistilled water, filtered and completed to 50 mL with bidistilled water.

2.4. Biological samples

Aliquots of the biological fluid (urine or plasma, obtained from a donor healthy male) were spiked with different VAR concentrations, treated with 0.1 mL of 70 % perchloric acid diluted to 10 mL with water, vortexed and centrifuged for 10 min at 13000 rpm. The supernatants were neutralized with NaOH to the desired pH value and the volume was completed to 25 mL with water.

2.5. Apparatus

Potentiometric measurements were carried out using UT61E Multimeter with RS-232 and USB interface (Uni-Trend Technology Co, China). JENWAY 3510 pH meter (England) with combined glass electrode was used for pH measurements.

2.6. Procedures

2.6.1. Sensor construction

The conducting carbon and silver/silver chloride tracks (5 × 35 mm) were printed on a polymeric sheet using graphite-based and silver-silver chloride inks, respectively as describes in details elsewhere [27]. The sensing matrix containing 2.0 mg 15-crown-5 ether (VII), 0.6 mg KTClPB, 360 mg *f*-PNPE, 6 mL tetrahydrofuran, 240 mg PVC and 10.0 mg MWCNTs was dropcasted on the carbon conducting track and left to dry at 50 °C for 24 h. The fabricated electrodes were used in potentiometric measurements after preconditioning in 10^{-3} mol L^{-1} VAR solutions for 20 min.

2.6.2. Sensor calibration

The fabricated sensors were calibrated by immersing the bielectrode strip in different VAR solutions covering the concentration range from 10^{-7} to 10^{-2} mol L^{-1} at 25 °C. Calibration graphs were achieved by plotting potential readings against drug concentration in logarithmic scale [28].

2.6.3. Sample analysis

Known increments of standard VAR solution were added to the sample solution and the electrode potentials for each increment were used to calculate VAR concentration in the sample solution [29].

Under the potentiometric titration, aliquots of the sample solution containing 0.49 to 4.89 mg VAR were titrated against standardized NaTPB solution

using the fabricated sensor as indicator electrode [30]. Potential readings were plotted against titrant volume and the equivalence points were estimated from the first derivative of the sigmoid-shape titration curves. The obtained recoveries were compared with the official method [2].

3. Result and Discussion

During the last decades, molecular recognition and inclusion complexation mechanism offer promising approaches for electrochemical sensing [31-35]. Pederson in 1967 [36], reported the synthesis of crown ethers (macrocyclic polyethers) and studied their application as complexing agents for alkali metals. Crown ethers have the general formula *n*-crown-*m*, where *n* is the number of atoms in the ring and *m* is the number of oxygen atoms. The lipophilic cavity of crown ethers macromolecule provides a micro environment which an appropriately sized nonpolar drug molecule, or more often nonpolar parts, can enter to form an inclusion complex [35, 37-39]. Inclusion complexes in which the organic substrates are incorporated into a host cavity, the predominant attractive host – guest interactions are the dipole – dipole, dipole –induced dipole, or induced dipole – induced dipole van der Waals forces. Overall, these macromolecules serve as reversible and reusable binding reagents that selectively extract the target analyte into the membrane. Such binding event creates the phase boundary potential at the membrane–sample interface. The selectivity of the complexes of crown ethers is based on the size of the substrate and the ring size and distribution of the donor atoms in the crown [31].

In a reported work [40], cyclodextrins were suggested to enhance the poor solubility, hasten the onset of action, and mask the unpleasant taste of VAR utilizing β-cyclodextrin (β-CD) and formulation of the inclusion complex as oral disintegrating tablets (ODTs). Based on this work, similar macromolecules such crown ethers were involved as a sensing ionophore for potentiometric determination of VAR.

3.1. Optimization of the sensor matrix compositions

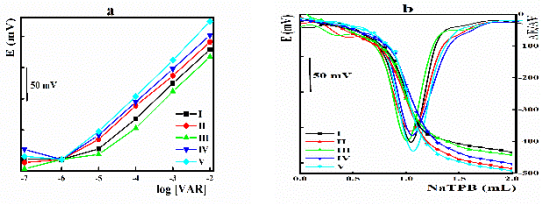
To achieve the highest sensor performance, parallel studies were performed including the effect of the nature of sensing ionophores, anionic sites, plasticizers and nanomaterials.

3.1.1. Effect of molecular recognition element

The effect of the sensing ionophore was tested via incorporation of different macromolecules families including cyclodextrins, crown ethers and calixarenes (I-XIII) within the electrode matrix. Dummy electrodes, fabricated without addition of sensing element, have sub Nernstian response (31.1±1.1 mV decade^{-1}), while those modified with different sensing ionophores showed improved Nernstian responses near the theoretical values (Fig. 1a-f). From different cyclodextrin derivatives, *β*-CD (II) and its methylated derivatives heptakis (*2,3,6-tr-O-methyl)-β*-cyclodextrin (IV) and heptakis (*2,3,6-tri-O-methyl)-β*-cyclodextrin (V) possesses the proper cavity sizes to incorporate the VAR molecule within their cavity; therefore their electrodes showed the highest potentiometric response (Nernstian slopes were 51.0±1.4, 53.0±1.0 and 55.6±1.8 mV decade^{-1}, respectively). Under potentiometric titration mode, the β-CDs (II and V) showed improved titration curves compared with other cyclodextrin derivatives (Fig. 1b).

Moreover, crown ether macromolecules with different rings size (ranged from 1.2 to 4.6 ^{o}A for VI to XI) were also tested as sensing elements for potentiometric determination of VAR via formation of VAR-CE inclusion complexes. 12-crown-4 ether (VI) with small ring size (1.2 ^{o}A) was improper for fitting the VAR within its cavity (Nernstian slope 52.8±0.3 mV decade^{-1}), while the next member 15-crown-5 ether (VII) was more efficient for fitting VAR within their cavity with higher stability and Nernstian slope values of 59.2±1.6 mV decade^{-1} (Fig.1 c& d). Higher crown ethers with larger ring cavity showed diminished sensitivities with slope value of 48.4±2.5 mV decade^{-1} for 30-crown-10 ether (XI) with cavity size 4.6 ^{o}A.

Following, the performances of sensors incorporated with *β*-CD compounds (V) and 15-crown-5 ether (VII) were compared with those containing calixarene derivatives (XII, XIII) (Fig. 1e & f). The obtained data revealed the superiority of the application of crown ether as sensing material compared with other tested ionophores.



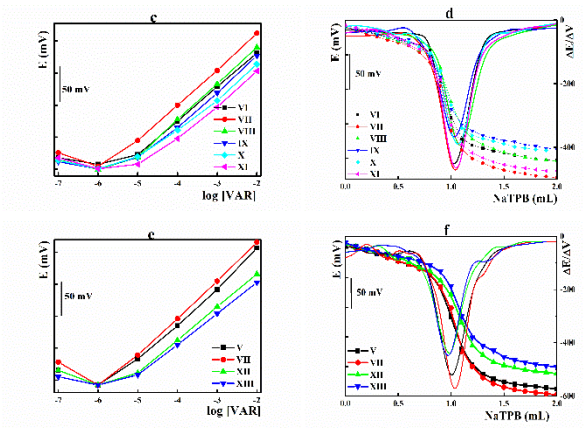


Fig. 1: a, c, e) Effect of the ionophores on sensor performance, b, d, f) potentiometric titration of 1.0 mL of 10^{-2} mol L^{-1} VAR with 10^{-2} mol L^{-1} NaTPB solution

The content of the sensing element within the electrode matrix plays a crucial rule on the electrode performance. Higher concentrations resulted in over saturation of the membrane matrix which hindering the complexation process, while lower concentration resulted in lower sensitivity. Herein, the content of CE (VII) within the sensing membrane matrix was varied from 0.0 to 4.0 mg. Nernstian responses increased from 31.1±1.1 for blank electrode to reach about 59.2±1.6 mV $decade^{-1}$ at 2.0 mg which was sufficient to obtain reasonable response.

3.1.2. Effect of ionic sites

Crown ethers belongs to the neutral carrier ionophores; therefore their potentiometric sensors can only operate in the presence ionic sites whose charges are opposite charge to the target analyte. Moreover, incorporation of such lipophilic ionic sites within the sensing matrix decreases the membrane resistance and enhances the interfacial ion-exchange kinetics at the membrane surface which reflected on a noticeable improvement of the selectivity and sensitivity of the sensor [41-43].

Lipophilic tetraphenylborate derivatives are usually selected as efficient ionic sites in potentiometric sensors selective for cationic drugs. Herein, in the absence of tetraphenylborates, the blank showed sub Nernstian response about 34.2±1.8 mV $decade^{-1}$ (Fig. 2a) compared to sensors modified with different tetraphenylborate derivatives (Nernstian response values were in the order 54.2±1.9, 58.7±0.9 and 60.4±0.7 mV $decade^{-1}$ for NaTPB, NaTFPB and KTClPB, respectively). The rule of the ionic sites liphophilicity was clearly noticed under potentiometric titration of VAR against NaTPB applying sensors contained different tetraphenylborate derivatives (Fig. 2b). The tetrakis-4-fluorophenyl borate (NaTFPB) and tetrakis-4-chlorophenyl borate (KTClPB) showed better titration curves indicated by high potential jump and potential change at the inflexion point. From different KTClPB concentrations within the electrode matrix, 0.6 mg was sufficient to obtain reasonable sensor performance.

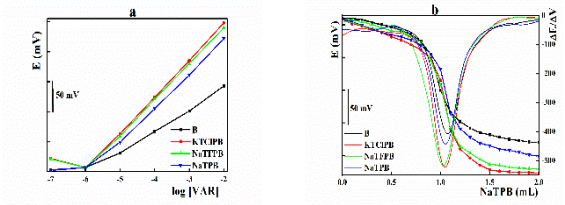


Fig. 2: Effect of the ionic sites on: a) sensor performance; b) potentiometric titration of 1 mL of 10^{-2} mol L^{-1} VAR with 10^{-2} mol L^{-1} NaTPB solution

3.1.3. Effect of membrane plasticizers

For polymeric membrane sensors, the nature of the plasticizer (membrane mediator) expressed by its dielectric constant (ε) governs mobility of the sensing element and the stability of the formed inclusion complex with the analyte **[**43-45]. To investigate the influence of the plasticizer on the sensor performance, four plasticizers with different dielectric constants were applied, namely, DOP, TCP, o-NPOE and f-PNPE (values of dielectric constant ε were 3.8, 17.6, 24 and 50, respectively) [46]. Sensors fabricated using highly polar plasticizers showed improved performances in the VAR concentration ranged from 1×10^{-6} to 1×10^{-2} mol L^{-1} (Nernstian slopes were 58.7 ± 0.4 and 61.1 ± 0.3 mV per decade for o-NPOE and f-PNPE, respectively) **(Fig. 3a)**. Other tested plasticizers showed lower Nernstian slope and narrow linear range (54.0 ± 0.8 and 56.8 ± 0.4 mV per decade for DOP and TCP, respectively).

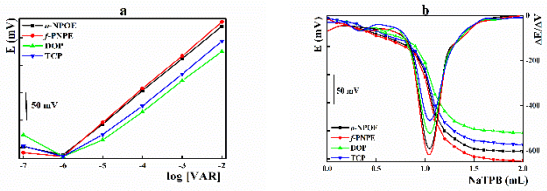


Fig. 3: Effect of the membrane plasticizer on: a) sensor performance; b) potentiometric titration of 1 mL of 10^{-2} mol L^{-1} VAR with 10^{-2} mol L^{-1} NaTPB solution

Similar concept was achieved under the potentiometric titration conditions of VAR against NaTPB with sensors containing different plasticizers **(Fig. 3b).** f-PNPE and o-NPOE with their high dielectric constant showed the highest potential jump compared with other tested plasticizers (the total potential change (ΔE) values were 253, 236, 225 and 204 mV with potential change at the inflexion point (dE/dv) values 580, 548, 485 and 430 for f-PNPE, o-NPOE, TCP and DOP, respectively.

3.1.4. Modification with nanomaterials

Nanomaterials with their unique characteristics promote the transduction of chemical signal to the electrical signal within the sensor matrix and improve the sensor performance [47, 48]. Aiming to improving the conductivity and hydrophobicity of the polymeric PVC membrane, different carbonaceous nanomaterials including multi-walled, single walled carbon nanotubes and graphene nanosheet were introduced into the electrode matrix (Fig. 4 a & b). Significant improvement was achieved via incorporation of nanomaterials indicated by higher Nernstian slope values compared with the blank PVC membrane. Under potentiometric titration, modification with MWCNTs showed the highest potential jump among the tested nanomaterials (ΔE values were 265, 262, 258 and 253 mV for MWCNTs, SWCNTs, rG and blank membrane respectively). The carbon nanotube content within the sensing cocktail was changed from 0 to 80 mg, and 10.0 mg was selected.

Moreover, nanomaterials with their high surface area improve transduction of the chemical signal to electrical signal and the matrix conductivity which improve in the response time of the sensors (the time required by electrode to achieve 90% of the total potential change) (Fig. 4 c). For practical application of commercial disposable sensors, the preconditioning time (time needed to achieve steady state potential reading 1.0 mV for a newly used electrode) is crucial important. Nanomaterials enhanced the sensing membrane hydrophobicity and in turn improve the preconditioning time compared with PVC membrane (less than 20 min) (Fig. 4 d).

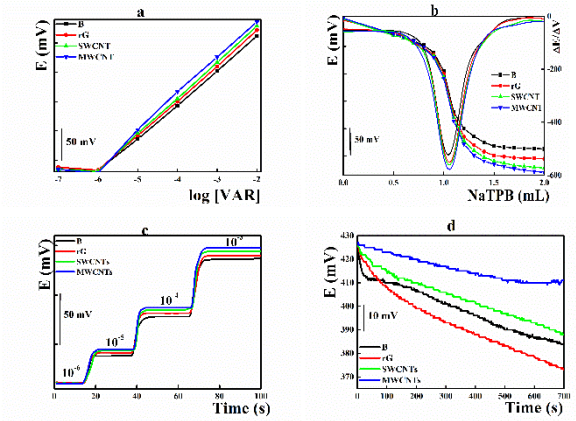


Fig. 4: Effect of the different nanomaterials on a) sensor performance; b) potentiometric titration of 1 mL of 10^{-2} mol L^{-1} VAR with 10^{-2} mol L^{-1} NaTPB solution; c) sensor response time; d) preconditioning time

3.2. Sensors performances

The performance of vardenafil sensor modified with 15-crown-5 ether (VII) as molecular recognition element and MWCNTS as transducer was evaluated according to the IUPAC recommendation [28]. Theoretical cationic Nernstian compliance of 62.1±0.8 mV $decade^{-1}$ in the vardenafil concentration range from 10^{-6} to 10^{-2} mol L^{-1} was obtained with electrode response time less than 4 s (Table 1).

The fabricated sensors can operate for a long shelf lifetime (24 weeks) during which the Nernstian slopes did not change significantly and the same electrode can operate contentiously up to more than 10 days without losing of performance. Moreover, high fabrication reproducibility, the average Nernstian slope values for ten printed sensors within the same batch was 61.5±0.8 mV $decade^{-1}$ with standard potential of (E_o) 385.4 ± 3.0 mV.

Table 1: Analytical performances of different VAR screen printed sensors

Sensors	SPE/CE (VII)	SPE/MWCNTs/ CE (VII)
Concentration range (mol L^{-1})	10^{-6}-10^{-2}	10^{-6}-10^{-2}
Slope (mV $decade^{-1}$)	61.1 ± 0.3	62.1 ± 0.8
R	0.9992	0.9995
LOD (mol L^{-1})	1.0×10^{-6}	8.0×10^{-7}
Response time (s)	8	<4
Preconditioning time (min)	120	<20
Shelf life time (week)	12	24

3.2.1. Effect of pH

For assaying of pharmaceutical compounds in their formulations and biological samples, the working pH range is one of the important operating factors. Herein, the effect of the supporting electrolyte pH on the electrode performance was investigated and the fabricated sensors showed table potentials readings (± 1.0 mV) in the working pH range from 2 to 5 while at higher pH values the potential was decreased dramatically with the formation of the deprotonated species and precipitation of VAR base (VAR pK_{a1} = 4.72; pK_{a2} = 6.21). [49].

3.2.2. Selectivity

The selectivity of the potentiometric sensor was defined by its ability to differentiate a particular target analyte in the presence of other interfering species [50]. In addition to the presence of excipients and additives in VAR pharmaceutical formulations, the selectivity of the fabricated is questionable. Matched potential method (MPM) was recommended for species with neutral compounds or different charges compared with the separate solution methods [50-52].

Table 2: Potentiometric selectivity coefficients of VAR - screen printed sensors

Interferent	-log $K_{A,B}$		
Li^+	2.70	Maltose	4.00
NH_4^+	2.15	Starch	4.23
Ca^{2+}	1.80	Sucrose	3.92
Mg^{2+}	2.12	Glucose	4.33
Ni^{2+}	1.60	Fructose	4.05
Co^{2+}	1.73	Glycine	2.14
Phosphate	3.60	Caffeine	2.65
Citrate	3.44	Cysteine	2.75

In the present study, the selectivity of the fabricated sensor toward VAR species was improved by incorporation of 15-crown 5-ether to the electrode matrix **(Table 2)**. The improved selectivity is due to formation of the VAR-CE inclusion complex.

3.3. Analytical applications

3.3.1. Potentiometric titration

For more accuracy and precision compared with direct potentiometric measurements, potentiometric titration techniques can be suggested for VAR determination using the fabricated sensor electrodes as indicator electrodes. Titration graphs **(Fig. 5)** were symmetrical with high potential jumps (from 192 to 265 mV) within VAR concentration from 0.49 to 4.89 mg.

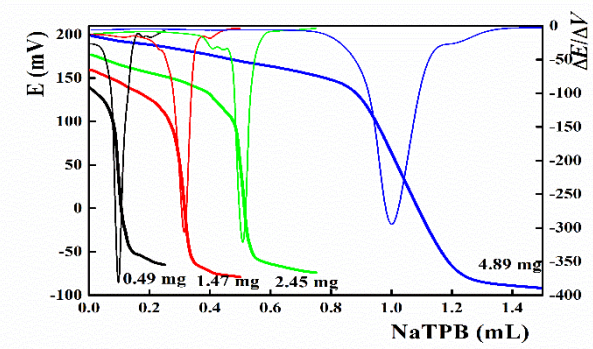


Fig. 5: Potentiometric titration of different VAR concentrations against NaTPB using VAR-screen printed sensors

It is noteworthy to mention that the presented electrode showed about four-fold sensitivity compared with those based on VAR-TPB ion associates or titration against N-bromosuccinimide (NBS) using Pt indicator electrode [20, 21]. The titration process was highly producible, for five successive titrations of 4.89 mg VAR, the titration curve shoed total change of potential 268.0 ± 1.4 mV with average recovery 101.05 ± 1.80%.

3.3.2. Sample Analysis

The achieved satisfactory sensitivity and selectivity of the fabricated sensors towards vardenafil suggested their application as s suitable tool for assaying of VAR in pharmaceutical dosage and biological samples **(Table 3)**. The excipients and additives present with VAR (microcrystalline cellulose, crospovidone, croscarmellose sodium, colloidal anhydrous silica or magnesium stearate) did not show a significant interference for assaying of vardenafil in pharmaceutical formulation and the achieved recoveries agreed with the official method.

Vardenafil is rapidly absorbed with absolute bioavailability of approximately 15% and metabolized predominantly by the hepatic enzyme CYP3A4, with contribution from the CYP3A5 and CYP2C isoforms. The major circulating metabolite results from desethylation at the piperazine moiety of vardenafil [1]. The metabolite still contains the tertiary amine active sit and can be assayed by the fabricated sensor. Thus, the proposed method can be suggested for determination of VAR in biological fluids as well as pharmaceutical formulations.

Table 3: Potentiometric determination of VAR in pharmaceutical preparations and biological fluids

Sample	Taken (mg) [ref.2]	Found			
		Standard addition		Potentiometric Titration	
		Recovery*	RSD	Recovery	RSD
Powerecta 20 mg, EVA Pharma	0.490	96.5	2.1	95.4	1.8
	1.470	98.2	1.8	97.1	1.42
	2.445	99.5	1.4	98.9	1.0
Rectivard 20 mg Marcyrl Co	0.490	97.0	2.2	96.1	2.0
	1.470	98.5	2.0	98.3	1.6
	2.445	100.0	1.7	99.8	1.1
Vardapex 10 mg, Multi Apex	0.490	95.4	2.4	94.8	2.1
	1.470	97.2	2.1	96.7	1.9
	2.445	98.4	1.9	97.5	1.8
Romantigra 20 mg, Global Napi	0.490	98.4	1.9	96.8	1.7
	1.470	100.2	1.5	98.2	1.1
	2.445	102.4	0.9	99.9	0.7
Spiked Plasma	0.490	94.2	3.2	95.1	2.9
	1.470	95.8	2.8	95.8	2.4
	2.445	96.2	2.1	97.0	1.8
Spiked Urine	0.490	95.2	2.9	96.3	2.1
	1.470	96.4	2.7	97.4	1.9
	2.445	98.3	2.4	99.0	1.7

- Mean recovery and relative standard deviations of five determinations

Conclusion

The present study described the fabrication of disposable vardenafil screen printed potentiometric sensors. From different tested molecular recognition elements, 15-crown 5-ether was selected in presence of MWCNTs as transducer in the electrode matrix. Ideal Nernstian response of 62.1±0.8 mV decade^{-1} was recorded in the concentration range from10^{-6} to 10^{-2} mol L^{-1} with a detection limit of 8.0×10^{-7} mol L^{-1}. Fast response time (< 4 s) and preconditioning time with long operational lifetime were the most promising futures of the fabricated sensors compared with previosuly reported VAR sensor. The fabricated sensor was also successfully applied for determination of vardenafil hydrochloride in pharmaceutical and biological samples with acceptable sensitivity compared with the official methods.

Conflicts of interest

There are no conflicts to declare.

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