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Novel Pioglitazone Nanomaterial Based Screen Printed Sensors

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Sensitive disposable potentiometric sensors for determination of pioglitazone hydrochloride (PIO) have been constructed. The fabricated screen printed electrodes (SPEs) are based on multi-walled carbon nanotubes - polyvinyl chloride (MWNT-PVC) composite and crown ether as sensing ionophore. Electrode matrices compositions were optimized referring the effect of nature and content the sensing ionophore, anionic sites, plasticizer and nanomaterial. Detailed investigation revealed that sensors incorporated with 15-crown-5 ether as sensing ionophore, sodium tetrakis (4-florophenyl) borate (NaTFPB) as anionic site and 2-fluorophenyl 2-nitrophenyl ether (*f*-PNPE) as membrane plasticizer showed the best electroanalytical performances. The fabricated electrodes worked satisfactorily in the PIO concentration range from 10^{-6} to 10^{-2} mol L^{-1} with Nernstian compliance of 61.05±0.5 mV $decade^{-1}$ and detection limit of 8×10^{-7} mol L^{-1}. Carbon nanotubes remarkably improved the potential stability and lifetime of the fabricated sensors. The sensors have been successfully applied for the potentiometric determination of PIO in pharmaceutical preparations under batch experiments and flow injection analysis (FIA) with acceptable average recoveries. The relative simple fabrication protocol of disposable sensor, high sensitivity and stability of the method represents a promising approach for drug quality control laboratories.

Keywords: Pioglitazone; Screen-printed potentiometric sensor; Carbon nanotubes; Flow injection analysis; Pharmaceutical analysis.

1. INTRODUCTION

The active moiety of pioglitazone (PIO) (5-[[4-[2-(5- ethylpyridin-2-yl) ethoxy] phenyl] methyl]-1,3-thiazolidine-2,4-dione) is a thiazolidinedione (Fig.1), a potent and highly selective agonist for the nuclear receptor, peroxisome proliferator-activated receptor gamma (PPAR-γ) [1]. PPARs are found in tissues like adipose tissue, skeletal muscle and liver, which are critical to insulin action. Activation of PPAR-γ modulates the transcription of a number of insulin-responsive genes involved in the control of glucose and lipid metabolism [2, 3].

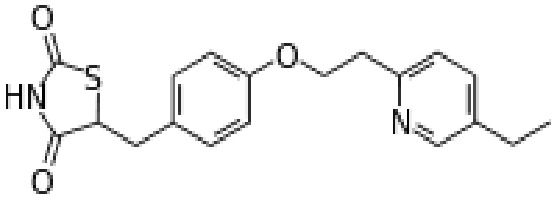

Figure 1. Chemical structure of PIO

Techniques like spectrophotometry [4-6], capillary electrophoresis [7], high-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC–MS) [8] have been used for PIO analysis, from which HPLC methods are the most extensively used [1, 9-11].

However, most of these methods are time-consuming and need sophisticated instruments and tedious sample pretreatment procedures. Owing to their advantages, potentiometric methods with ion selective electrodes (ISEs) are of choice since they possess the advantages of simplicity and accuracy without separation or pretreatment procedures [12-15]. Mostafa et al. [16] constructed a PVC membrane sensor incorporated ion association complexes of PIO and sodium tetraphenylborate (NaTPB), phosphomolybdic acid (PMA) or phosphotungstic acid (PTA) as electroactive materials for direct determination of PIO in the concentration range 1×10^{-2} to 10^{-6} mol L^{-1}. Similar membrane electrodes using the ion association complexes between PIO with either NaTPB or ammonium reineckate (RNC) counter ions was applied for the determination of pioglitazone HCl in the presence of its acid degradant or metformin HCl in tablets and plasma [17, 18]. Badawy et al. [19] quantified PIO and other antidiabetic drugs (rosiglitazone, glimepiride and glyburide) in their pharmaceutical preparations using carbon paste (CPE) - and PVC membrane electrodes. The preparation of these ion-selective electrodes for the potentiometric determination of the drug is based on the construction of a 10% standard drug-ion pair with reineckate or tungstophosphate as electroactive materials. Potentiometric sensors incorporated with ion-pair associates are generally plagued by limited selectivity and their applications are restricted to more challenging matrices; therefore a more selective molecular recognition component is clearly required.

Ionophore incorporated sensors are well-established analytical tools routinely used for the selective and direct measurement of different analyts in complex biological and environmental samples [20-22]. The vital component of such sensors is the ionophore, which defining the selectivity and sensitivity of the fabricated sensors. Different types of supramolecules sensing ionophore, like calixarenes [23], cyclodextrins [24-26] and crown ethers [27-30] have been used. Few reports on the use of these molecular receptors as sensing material for drug analysis are available. Crown-ether containing PVC plasticized membrane had been shown to have sensitivity toward primaquine [31], promethazine [32], mexiletine [33] and dopamine [34].

Traditional PVC and CPEs are inconvenient for commercialization taking into account their construction, size and necessity of conditioning before measurements. Screen printing seems to be one of the most promising technologies allowing sensors to be produced on a large-scale with the advantages of optimized manufacturing repeatability, long shelf-lifetime and application the field measurements with portable small instruments [35-37]. Moreover, nanotechnology brought important

and challenging opportunities for sensor construction and developing new electrochemical approaches. Only two decades after their introduction, Carbon nanotubes (CNTs) received enormous attentions in the fields of electrochemistry and analytical electrochemistry [38-40]. Carbon nanotubes (CNTs) are excellent sensor materials due to their high conductivity, chemical inertness and large surface area. Incorporation of CNTs in electrode matrices improves the conductivity and transduction of the chemical signal to electrical signal, which in turn improved the dynamic working range and response time [41].

The aim of the present work is to introduce disposable screen printed electrodes (SPEs) incorporated with carbon nanotubes and crown ethers as potentiometric sensors for pharmaceutical analysis. The developed methods are simple, rapid, accurate, precise and sensitive for the determination of pioglitazone in various dosage forms under batch and FIA conditions.

2. EXPERIMENTAL PART

2.1. Reagents

All reagents were of the analytical grade and bidistilled water was used throughout the experiments. Different cyclic macromolecules were tested as sensing ionophores including; native α- (**I**), β- (**II**) and γ-cyclodextrin (**III**) (Sigma), heptakis (2,6-di-O-methyl)-β-cyclodextrin (**IV**, Aldrich), heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin (**V**, Aldrich), 12-crown-4 ether (**VI**, Fluka), 15-crown-5 ether (**VII**, Fluka), 18-crown-6 ether (**VIII**, Fluka), calix[4]arene (**IX**) and calix[8]arene (**X**) (Aldrich). Sodium tetraphenylborate (NaTPB, Fluka), sodium tetrakis (4-fluorophenyl) borate (NaTFPB, Fluka) and potassium tetrakis (4-chlorophenyl) borate (KTCPB, Fluka) were used as anionic sites.

The tested electrode plasticizers were as following; *o*-nitrophenyl octyl ether (*o*-NPOE, Sigma), 2-fluorophenyl 2-nitrophenyl ether (*f*-PNPE, Fluka), dioctylphthalate (DOP, Sigma), dioctylsebacate (DOS, Avocado) and tricresylphosphate (TCP, Fluka). 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 preparation of the printing ink. Different carbon nanomaterials including: multiwall carbon nanotubes (MWCNTs, Aldrich), single wall carbon nanotubes (SWCNTs, Aldrich), and carbon nano powder (CNP, Sigma) were used. MWCNTs were functionalized via introducing of carboxylic acid moieties onto its surface through oxidation in acidic medium. Carbon nanotubes were dispersed in 2.0 mol L^{-1} nitric acid solution for 24 h at 25 ^{o}C, washed afterwards with deionized water and the functionalized multiwall carbon nanotubes (FMWCNTs) were dried at 75 ^{o}C [42].

2.2. Authentic Samples

Pioglitazone authentic sample was kindly provided from National Organization for Drug Control and Research, Giza, Egypt. The purity of the samples was found to be 100.05± 0.25% according to the official HPLC method [43]. The stock solution of 1×10^{-2} mol L^{-1} was prepared by

dissolving the appropriate amount of PIO in 100 mL of aqueous acidic solution (0.1M HCl). Other working solutions (1.0×10^{-3} to 1.0×10^{-7}) were prepared by dilution with bidisillied water.

2.3. Pharmaceutical preparations

Pioglitazone pharmaceutical formulations including Glustin (Lilly, Egypt, 15 mg/tablet) and Hi-glitazone (Hi Pharm, Egypt, 30 mg/tablet) were obtained from local drug stores. Ten tablets of each pharmaceutical preparation were accurately weighed and powdered. An accurate weight equivalent to two tablets was transferred into a beaker containing 10^{-1}mol L^{-1} HCl with sonication for 15 min. The sample was filtered, completed to 25 mL and analyzed using the proposed method and compared with the official method.

2.4. Apparatus

Potentiometric measurements were carried out using a 692-pH meter (Metrohm, Herisau, Switzerland). A single line flow injection system, composed of four channel peristaltic pump (MCP Ismatec, Zurich, Switzerland), sample injection valve (ECOM, Ventil C, Czech Republic) and continuous flow cells adapted for screen printed electrodes [44], was constructed for FIA measurements. 46-Range Digital Multimeter (Radioshack, China) with PC interface was used for potentiometric measurements in case of FIA and response time measurements.

2.5. Procedures

2.5.1. Sensor construction

The potentiometric bielectrode strips (with dimensions 5 × 35 mm) were printed on a PVC sheet using silver-silver chloride and graphite-based pastes for reference and working electrodes, respectively [45]. The ion-sensing cocktail, containing 3.0 mg 15-crown-5 ether (**VII**), 1.0 mg NaTFPB, 360 mg *f*-PNPE and 240 mg PVC dissolved in 6 mL tetrahydrofuran, was printed on the surface of the graphite/PVC conducting track and left to dry at 50 ^{o}C for 24 h. In alternative sensing cocktail, 20 mg of MWCNTs were added to the aforementioned matrix, sonicated for 30 min and typically printed on the conducting carbon track. All the fabricated electrodes were directly used in potentiometric measurements after preconditioning in 10^{-3} mol L^{-1} PIO solutions for 10 min.

For comparison, CGEs were constructed using graphite rod (spectroscopic grade, 3 mm diameter and 10 mm long) following the procedures described in details elsewhere [26]. The polished and cleaned electrodes were dipped in the above matrix cocktail for 15 times, after each the solvent was evaporated using air gun. The coated electrodes were left to dry at room temperature and preconditioned in 10^{-3} mol L^{-1} PIO solution for 2 h before use.

2.5.2. Sensor calibration

For batch measurements, the developed sensors were calibrated by immersing the bielectrode strip in different PIO solutions covering the concentration range from 10^{-7} to 10^{-2} mol L^{-1} at 25 ^{o}C. The potential readings were recorded and plotted against drug concentration in logarithmic scale [46].

Operating FIA measurements, 200 µL of freshly prepared drug solutions covering the range from 10^{-6} to 10^{-2} mol L^{-1} were injected in 10^{-1}mol L^{-1} HCl flowing stream with flow rate of 12.6 mL min^{-1}. The corresponding peak heights were recorded and used to draw the calibration graphs.

2.5.3. Potentiometric determination of pioglitazone in pharmaceutical preparations

Pioglitazone contents in different pharmaceutical preparations were potentiometrically determined using the developed sensors by potentiometric titration and FIA measurements. Aliquots of the sample solutions containing 3.56 to 24.92 mg of PIO were titrated against standardized NaTPB solution [47] using the fabricated PIO bielectrode strip as indicator electrode. Potential readings were plotted against the titrant volume to estimate the end point.

Under FIA conditions, 200 µL of the sample solutions were injected in the flowing stream at the optimum measuring conditions and the peak heights were compared with those obtained from injecting standard solutions of the same concentration. The obtained recoveries were compared with the official method for the cited drug.

3. RESULTS AND DISCUSSION

Early 1970, molecular recognition in biological systems attracted synthetic chemists. In 1967, crown ethers (macrocyclic polyethers) were synthesized by Pederson (Nobel Prize for Chemistry, 1987) who also studied their use as complexing agents for alkali metal and other cations [48, 49]. The general method of naming crown ethers is to use the form *n*-crown-*m*, where *n* is the number of atoms in the ring and *m* is the number of oxygen atoms. Crown ether structure exhibits a conformation with a so-called hole capable of trapping guest by coordination with a lone pair of electrons on the oxygen atoms. The stability of these complexes depends on the size of the ion relative to the cavity available in the ring of the particular crown ether [27-30]. Many examples of chiral crown ethers are published but only a few reports on the use of these molecular receptors as sensing material for drug analysis are available [31-34].

Application of crown ethers as carriers for pioglitazone- sensitive electrode is based on their strong affinity towards primary amines forming host-guest complexes. The size of the cavities provided by the crown ether is of importance and better electrode performance is to be expected for those carriers with cavity size sterically matching the PIO molecule. Crown ethers showed different cavity size ranging from 1.2A for 12-crown-4 to 3.4 A for 21-crown-7. Overall, these ionophores 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 [50].

In a reported work [51], cyclodextrin was suggested to enhance aqueous solubility of PIO through formation of PIO-cyclodextrin inclusion complexes. Based on this work, different macromolecules including cyclodextrin, crown ethers and calixarenes can be tested as a sensing ionophore for potentiometric determination of PIO.

3.1. Optimal sensor matrices compositions

In preliminary experiments, ten different cyclic compounds were tested as sensing material including; native α- (**I**), β- (**II**) and γ-cyclodextrin (**III**), heptakis (2,6-di-O-methyl)-β-cyclodextrin (**IV**), heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin (**V**), 12-crown-4 (**VI**), 15-crown-5 (**VII**), 18-crown-6 (**VIII**), calix[4]arene (**IX**) and calix[8]arene (**X**). From different tested cyclodextrins, native β-CD showed the best performance towards PIO (Fig.2 a), while 15-crown-5 was the best from different crown ether with Nernstian slope value 59.5 ± 1.07 mV decade^{-1} in the concentration range from 10^{-6} to 10^{-2} mol L^{-1} (Fig. 2b). Moreover, the content of 15-crown-5 within the electrode matrix was varied from 0 to 5.0 mg and incorporation of 3.0 mg of the selected ionophore was sufficient to obtain reasonable response (Fig. 2c).

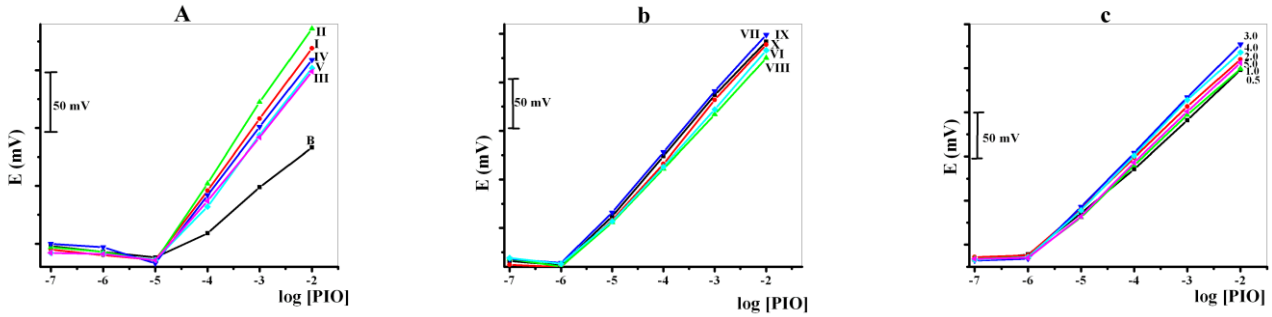

Figure 2. Effect of sensing ionophore nature and content on pioglitazone electrode performance.

Crown ethers behave as neutral carrier ionophores; therefore their sensors function only in presence of ionic additives [27, 30]. Lipophilic ionic sites promote the interfacial ion-exchange kinetics and decrease the bulk resistance by providing mobile ionic sites in the electrode matrix [52, 53]. Blank electrodes (without ionic sites) showed poor Nernstian response (about 36.4± 0.7 mV decade^{-1}) compared with those modified with different ionic sites (Fig. 3a). From different teteraphenyl borate derivatives, NaTFPB gave the best electrode characteristic (Nernstian slope 60.0 ± 0.7 mV decade^{-1}). In addition, operating the potentiometric titration using electrodes modified with the aforementioned ionic sites also sustained the priority of NaTFPB compared with other tested ionic sites (Fig. 3b). Furthermore, different amounts of NaTFPB (from 0 to 5 mg) were added to the electrode matrix and 1.0 mg was selected.

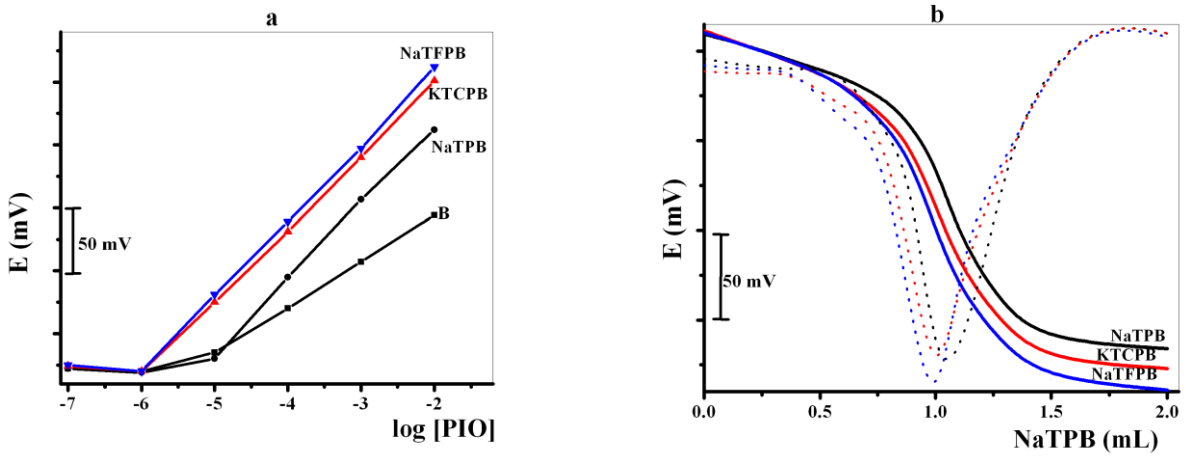

Figure 3. Effect of ionic sites on pioglitazone electrode performance; where dashed lines are for the first derivatives of the titration curves.

The influence of the membrane plasticizer on electrode performance was studied using five plasticizers having different dielectric constant, namely; *f*-PNPE, *o*-NPOE, TCP, DOS and DOP (ε = 50, 24, 17.6, 5.2 and 4.7, respectively). Application of the less polar plasticizers (Fig. 4 a) produced electrode with lower Nernstian slopes (53.7 ± 2.0, 56.5 ± 2.1, and 57.6 ± 2.3 mV decade^{-1}, for DOS, DOP and TCP, respectively). Contrary, *o*-NPOE and *f*-PNPE improved the electrode sensitivity (slope values were 60.1 ± 1.1 and 61.0 ± 0.5 mV decade^{-1}, respectively) which may be attributed to the presence of aromatic rings within the plasticizer structure can enhance the solubility of the ionophore and the formed PIO-15-crown-5 inclusion complex within the electrode matrix [54, 55]. Potentiometric titrations were carried out using sensors fabricated with aforementioned plasticizers. Different total potential changes and inflection of potential at the end point was achieved depending on the polarity of plasticizer (Fig. 4b). The obtained ΔE values were 275, 242.5, 176, 171, 167 mV for the tested plasticizers with highest inflection at the end point for *f*-PNPE which can be explained on the bases of plasticizer [56].

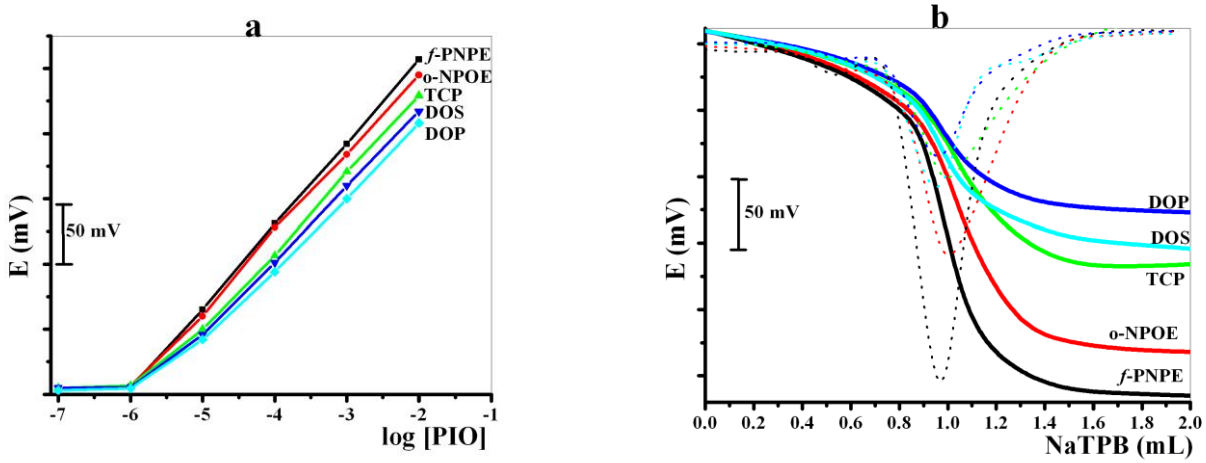

Figure 4. Effect of plasticizer on pioglitazone electrode performance; where dashed lines are for the first derivatives of the titration curves.

Incorporation of nanomaterials in the electrode matrix improves the conductivity and transduction of the chemical signal to electrical signal, which in turn improved the dynamic working range and electrode response time [57, 58]. Different carbon nanomaterials were added to the electrode matrix including: MWCNTs, FMWCNTs, SWCNTs, and carbon nano powder. The obtained results revealed the superiority of MWCNTs (Nernstian slopes was 61.8 ± 1.0 mV decade^{-1}) compared with other tested carbon nanomaterials. FMWCNTs showed lower slope values (51.2 ± 1.6 mV decade $^{-1}$) due to the presence of carboxylic group that disturb the dispersion of CNTs within the PVC matrix and adsorption of interfering species on the electrode surface. Furthermore, different amounts of MWCNTs (ranging from 0 to 270 mg) were added to the electrode matrix, and incorporation of 20 mg was the most suitable.

3.2. Sensor performances

Performances of the fabricated sensors compared with traditional coated graphite electrode (CGEs) prepared with the same electrode matrix were evaluated according to the IUPAC recommendation (Table 1). Screen printed electrodes modified with MWCNTs showed the best performance compared with other electrodes (Nernstian slope and detection limit values were 62.0±1.0 mV decade^{-1} and 8.0×10^{-7} mol L^{-1}, respectively).

The dynamic response times of the fabricated sensors were tested by measuring the time required to achieve a steady state potential (within ±1 mV) after sudden increase in the PIO concentration. Sensors modified with MWCNTs and 15-crown-5 showed fast response time (about 4 s) compared with 6 and 9 s for other sensors.

Table 1. Analytical performances [a] of various PIO sensors

Sensors	SPE/CNT	SPE	CGE/CNT
Concentration range (mol L^{-1})	10^{-6}-10^{-2}	10^{-6}-10^{-2}	10^{-5}-10^{-2}
Slope (mV decade^{-1})	62.0±1.0	60.8±0.9	58.9±1.8
R	0.99967	0.99964	0.99861
LOD (mol L^{-1})	8.0×10^{-7}	1.0×10^{-6}	2.7×10^{-6}
Response time (s)	< 4	6	9
Preconditioning time (min)	<10	15	90
Shelf lifetime (week)	24	18	4

[a] Results are the average of five different calibrations..

One disturbing drawback of solid contact electrodes was the potential drift and the poor adhesion of the electroactive membrane to the metal substrate [59]. Such phenomena, can explained on the basis of water and ions penetration through the sensing membrane with formation of undefined layer between sensing membrane and conductor. Herein, the sensing layer contain the same polymer matrix of the conducting track, and co-polymerization of the two PVC matrices (sensitive membrane and conducting track) during electrode fabrication will hinder formation of such internal water layer

and improve the electrode potential stability. SPEs showed high potential stability of as 10 min preconditioning time was sufficient to get stable potential compared with 90 min for CGEs (Table 1). Incorporation of MWCNTs enhances the hydrophobicity of the membrane, which contributes to the more stable potential signal by elimination of undesirable water layer at the interface [60].

Due to the solid nature of the electrode configuration (absence of the internal filling solution), the fabricated sensor showed useful shelf lifetime at 4^{o}C of 24 weeks during which the Nernstian slopes did not change significantly (±1 mV decade^{-1}). Even though the use of the SPEs allows a single use of the biosensor, it can be reliably applied up to twenty times without significant losses of the sensitivity. In addition, screen printing technology offers the advantages of high fabrication reproducibility. The average Nernstian slope values for ten printed electrodes within the same batch were 60.1 ± 0.5 mV decade^{-1}.

In an approach to understanding the impact of pH on the electrode response, the elecetrode potential was measured at particular concentration of the pioglitazone solution (1.0×10^{-3} mol L^{-1}) in different pH values from 2 up to 10 using NaOH or HCl solutions for pH adjustment. The electrode potential remained constant despite the pH changes in the range of 2.0 to 5.5, indicating the applicability of this electrode in such pH range. At higher pH values (>5.5), the potential decreased due to the gradual increase in the concentration of the unprotonated POI resulting in the precipitation of PIO base.

In pharmaceutical analysis, it is important to test the sensor selectivity towards the target analyt in presence of excipients which are usually present in pharmaceutical formulations, such as glucose, starch, talc, lactose, sucrose. Applying Matched Potential Method (MPM) [61], the fabricated sensors showed high selectivity toward PIO in the presence of other interferents, additives and fillers introduced in pharmaceutical formulations (Table 2).

Table 2. Selectivity coefficients for PIO sensors under batch and FIA conditions

Interferent	-log $K_{A,B}$				
	Batch	FIA		Batch	FIA
NH_4^+	3.10	4.3	Glucose	3.52	
K^+	2.10	3.5	Fructose	3.18	
Na^+	1. 20	3.40	Maltose	4.20	
Li^+	1.46	3.70	Lactose	3.80	
Ca^{2+}	2.37	3.52	Starch	3.80	
Mg^{2+}	2.90	3.42	Citric acid	4.20	
Mn^{2+}	2.72	3.70	Ascorbic acid	3.81	
Cu^{2+}	3.10	3.30	Glycine	3.45	
Cd^{2+}	2.45	3.15	Alanine	3.15	

3.3. Potentiometric titration

In addition to the direct potentiometric determination of PIO, the fabricated disposable sensors can applied as indicator electrodes in potentiometric titration of PIO with NaTPB. Under the optimum conditions, titration curves were symmetrical with well-defined potential jumps (about 275 mV)

allowing the determination of 3.56 mg PIO. Between day assays was tested by performing three different titration runs on three different days. In addition crown ether based sensors presented in this work showed better titration curve compared to those modified with PIO-TPB ion pair [16] regarding the total potential change and the inflection at the end point.

3.4. Electrode response under FIA conditions

Flow injection analysis (FIA) becomes a wide spread methodology characterized by versatility, ease of automation and high sampling frequency [62, 63]. In the present work, a home-made Perspex flow through cell was used, providing low dead volume, fast response, good washing characteristics and ease of construction [44]. Operating FIA measurements, 200 μL of freshly prepared drug solutions covering the range from 10^{-6} to 10^{-2} mol L^{-1}, were injected in 10^{-1}mol L^{-1} HCl flowing stream with flow rate of 12.6 mL min^{-1}. The corresponding peak heights were recorded and used to draw the calibration graphs. Linear calibration graphs were obtained in the concentration range from 10^{-5} to 10^{-2} mol L^{-1} with Nernstian slopes of 58.5 ± 0.8 mV $decade^{-1}$ and sampling output of 90 sample h^{-1} (Fig. 4) Reproducibility was evaluated from repeated 10 injections of 200 μL of 10^{-3} mol L^{-1} PIO solution and the average peak heights were found to be 160.0 ± 1.4 mV.

FIA is viewed as a well efficient mean for improving the performance characteristics of ISEs as the action of the flowing stream continuously cleans the electrode surface and the transient nature of the signal may help to overcome the effect of interfering ions. Under FIA conditions, the selectivity coefficients were calculated according to the separate solution method (SSM) [64], based on potential values corresponding to the peak heights for the same concentrations of the drug and the interferents. The fabricated electrodes were highly selective which and the selectivity coefficients were improved compared with batch measurements (Table 2).

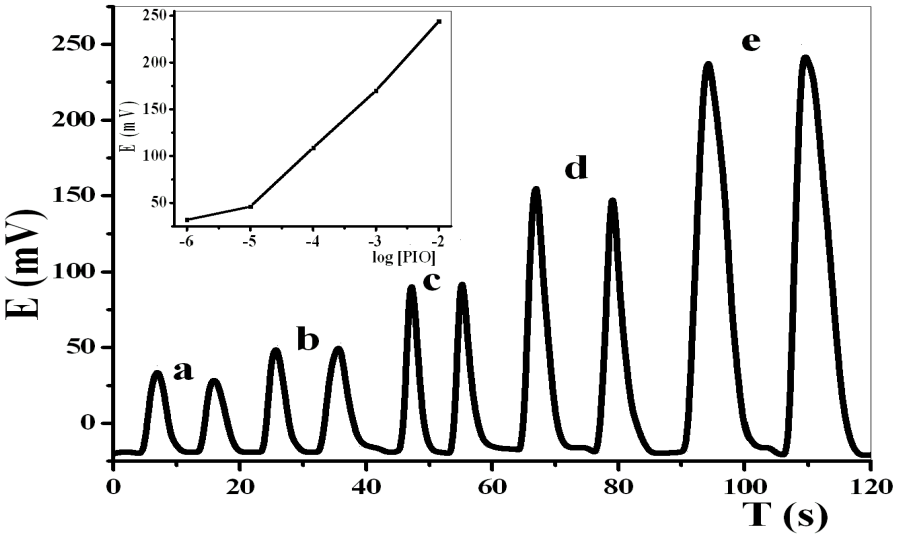

Figure 4. A) FIA potentiometric determination of PIO using 15-crown-5 ether based sensors: (a) 10^{-6}, (b) 10^{-5}, (c) 10^{-4}, (d) $10^{-3}$and (e) 10^{-2} mol L^{-1}.

3.5. Analytical applications

The fabricated sensors can be applied for PIO determination its pharmaceutical formulations under FIA and potentiometric titration methods. The obtained average recoveries showed agreement between the proposed and official PIO method [43] (Table 3).

Table 3. Determination of PIO in pharmaceutical preparations and statistical comparison to the official method

Method		Pharmaceutical Preparation									
	Taken (mg)	Glustin (15 mg/tablet)					Hi-glitazone (30 mg/tablet)				
		Found (mg)	Recovery	SD	t-Test	F-Test	Found (mg)	Recovery	SD	t-Test	F-Test
Potentiometric titration [n=6]	3.560	3.60	101.12	0.75	1.484 *[2.262]	1.858 *[6.26]	3.55	99.72	0.94	0.902 *[2.262]	1.570 *[6.26]
FIA [n=10]	0.360	0.362	100.56	0.68	1.172 *[2.160]	1.525 *[6.00]	0.357	99.17	0.85	1.071 *[2.160]	1.284 *[6.00]
Official HPLC Method [n=5]	0.360	0.367	101.94	0.55			0.361	100.28	0.75		

* The values in the parenthesis are the corresponding theoretical values of t and F at P=0.05

4. CONCLUSION

Based on the electrode performance studies, 15-crown-5 ether was selected as a suitable sensing ionophore in fabrication of pioglitazone potentiometric screen printed disposable sensor. A wide linear range of concentration range from 10^{-6} to 10^{-2} mol L^{-1}, low detection limit of 8.0×10^{-7} mol L^{-1} and fast response time of <4s are characterizations of the proposed sensors. A comparison between the proposed pioglitazone selective electrode and those reported in the literature based on ion-pair as sensing material, revealed some superiority in terms of the easier fabrication protocol, improved response time and lifetime. Improved sensitivity and selectivity was achieved via application of crown ether as molecular host–guest recognition element in combination with MWCNTs as ion-to-electron-FIA allows high sampling output with the possibility for incorporation in routine analysis for drug quality control. High selectivity and rapid response make these electrodes suitable for measuring the concentration of PIO in a variety of samples without the need for pretreatment or manipulation steps.

References

1. N. Satheeshkumarn, S. Shantikumar, R.Srinivas, *J. Pharm. Anal.* 4 (2014) 295.
2. G. Belcher, C. Lambert, G. Edwards, R. Urquhart, D.R. Matthews, *Diabetes Res. Clin. Pract.* 70 (2005) 53.
3. J.M. Olefsky, *J. Clin. Invest.* 106 (2000) 467.
4. S.S. Kumar, Y. Krishnaveni, G. Ramesh, *Int. J. Curr. Pharm. Res.* 4 (2012)113.

5. L. Adhikari, S. Jagadev, S.Sahoo, S Sahoo, P.N Murthy, U.S Mishra, *Int. J. Chem. Tech. Res.* 4 (2012) 625.
6. M. Amanlou, M. Zarei-Ghobadi, M.K. Rofouei, S. Saremi, A. Kebriaeezadeh, *J. Chem.* 7 (2010) 915.
7. B. Jamal, G.C. Theill, Lise-Lotte Sørensen, *J. Chromat. A* 1049 (2004) 183.
8. V.K. Karra, N.R. Pilli, J.K. Inamadugu, J.V. Rao, *J. Pharm. Anal.* 2 (2012)167.
9. A. Jedlicka, J. Klimes, T. Grafnetterova, *Pharmazie* 59 (2004)178.
10. S. Sharma, M.C. Sharma, S.C. Chaturvedi, *J. Optoelectron. Biomed. Mater.* 1 (2010)17.
11. G.R.K. Reddy, V.S.N. Rao, *J. Global Trends Pharm. Sci.* 3 (2012) 584.
12. V. V. Cosofret and R.P. Buck, Pharmaceutical Applications of Membrane Sensors, CRC Press, Boca Raton, FL, 1992.
13. K. Vytras, Advanced Instrumental Methods of Chemical Analysis, 1st ed., Ellis Horwood, Chichester, UK, 1993.
14. B. Singhal, *World J. Chem. 6* (2011) 59.
15. M. Gumustas, A.S. Ozkan, *Open Anal. Chem. J.* 5 (2011) 1.
16. G.A.E. Mostafa, A. Al-Majed, *J. Pharm. Biomed. Anal.* 48 (2008) 57.
17. M. El-Ghobashy, A. Yehia, A. Mostafa, *Anal. Lett.* 42 (2009)123.
18. F. Faridbod, M.R. Ganjali, E. Nasli-Esfahani, B. Larijani, *Int. J. Electrochem. Sci.* 5 (2010) 880.
19. S. Badawy, M.A. El-Ries, I.M. Mahdi, *Anal. Sci.* 25 (2009) 1431.
20. J.A. Cox, M.E. Tess, T.E. Cummings, *Rev. Anal. Chem.* 15 (1996) 173.
21. J. Zen, A.S. Kumar, D. Tasi, *Electroanal.* 15 (2003) 1073.
22. M. Trojanowicz, M. Weislo, *Anal. Lett.* 38 (2005) 523.
23. M.M. Zareh, E. Malinowska, *J. AOAC Int.* 90 (2007) 147.
24. E. Khaled, H.N.A. Hassan, Gehad G. Mohamed, Fahem A. Ragab, A.A. Seleim, *Int. J. Electrochem. Sci.* 5 (2010) 448.
25. E. Khaled, M.S. Kamel, H.N.A. Hassan, H.Y. Aboul-Enein, *J. Electroanal. Chem. 661* (2011) 239.
26. M E.M. Hassouna, G.A. Zaki, E. Khaled, *Int. J. Electrochem. Sci.* 10 (2015) 162.
27. H.S. Nalwa, *Nanostructured Materials and Nanotechnology*, ACADEMIC PRESS, USA, 2002.
28. F. Faridbod, M.R. Ganjali, R. Dinarvand, P. Norouzi, S. Riahi, *Sensors* 8 (2008) 1645.
29. G.W. Gokel, W.M. Leevy, M.E. Weber, *Chem. Rev. 104 (*2004) 2723.
30. J.M. Bradshaw, R.M. Izatt, A.V. Bordunov, C.Y. Zhu, J.K. Hathaway, in *Comprehensive Supramolecular Chemistry*, Gokel, G. W.; Ed; Pergamon: Oxford, 1996; Vol. 1, p 35
31. B.B. Saad, Z.A. Zahid, S.A. Rahman, M.N. Ahmad, A.H. Husin, *Analyst* 117 (1992) 1319.
32. A.K. Hassan, B.B. Saad, S.A. Ghani, R. Adnan, A. Abdul Rahim, N. Ahmad, M. Mokhtar, S.T. Ameen, S.M. Al-Araji, *Sensors* 11 (2011) 1028.
33. T. Katsu, Y. Mori, K. Furuno, Y. Gomita, *J. Pharm. Biomed. Anal.* 19 (1999) 585.
34. A.M. Othaman, N.M.H. Rizka, M.S. El-Shahawi, *Anal. Sci.* 20 (2004) 651.
35. J. P. Hart, A. Crew, E. Crouch, K. C. Honeychurch, R. M. Pemberton, *Anal. Lett.* 37 (2004) 789.
36. O. D. Renedo, M. A. Alonso-Lomillo, M. J. Martinez, *Talanta* 73 (2007) 202.
37. M. Li, Y. Li, D. Li, Y. Long, *Anal. Chim. Acta* 743 (2012) 31.
38. A. Merkoc, M. Pumera, X. Lopis, B. Perez, M. del Valle, S. Alegret, *Trends Anal. Chem.* 24 (2005) 826.
39. X. Ji, R. O. Kadara, J. Krussma, Q. Chen, C. E. Banks, *Electroanal.* 22 (2010) 7.
40. P. Yanez-Sedeno, J. Riu, J. M. Pingarron, F. X. Rius, *Trends Anal. Chem.* 29 (2010) 939.
41. Z. Spitalsky, D. Tasis, K. Papagelis, C. Galiotis, *Prog. Polym. Sci.* 35 (2010) 357.
42. B. Perez, M. Pumera, M. Del Valle, A. Merkoci, S. Alegret, J. Nanosci. Nanotechnol. 5 (2005) 1694.
43. British Pharmacopiea, Stationary Office, London, 7 th Edn. Vol II, 2014.
44. E. Khaled, H. N. A. Hassan, G. G. Mohamed, A. A. Seleim, *Drug Testing and Anal.* 2 (2010) 424.

45. E. Khaled, M. S. Kamel, H. N. A. Hassan, H. Abdel-Gawad, H. Y. Aboul-Enein, *Talanta* 119 (2014) 467.
46. R.P. Buck, E. Lindner, *Pure Appl. Chem.* 66 (1994) 2527.
47. K. Vytras, *Ion Select. Electrode Rev.* 7 (1985) 77.
48. C.J. Pedersen, *J. Am. Chem. Soc*. 89 (1967) 7017.
49. C.J. Pedersen, *Science* 241 (1988) 536.
50. E. Weber, Supramolecular Chemistry II-Host Design and Molecular Recognition, Springer-Verlag Berlin Heidelberg, 1995.
51. V. Pandit, R. Gorantla, K. Devi, *J. Young Pharm.* 3 (2011) 267.
52. E. Bakker, E. Pretsch, Anal. Chim. Acta 309 (1995) 7.
53. M. Telting-Diaz, E. Bakker, Anal. Chem. 73 (2001) 5582.
54. E. Bakker, P. Buhlmann, E. Pretsch, Electroanalysis 11 (1999) 915.
55. W.E. Morf, The Principles of Ion-Selective Electrodes and Membrane Transport, Elsevier, New York, 1981.
56. K. Vytras, J. Kalous, J. Jezkova, Egypt. J. Anal. Chem. 6 (1997) 107
57. Z. Spitalsky, D. Tasis, K. Papagelis, C. Galiotis, *Prog. Polym. Sci.* 35 (2010) 357.
58. J. Guo, Y. Chai, R. Yuan, Z. Song, Z. Zou, *Sensor Actuat.* B 155 (2011) 639.
59. B.P. Nikolskii, E.A. Materova, *Ion-Sel. Electrode Rev.* 7 (1985) 3-39.
60. J. Bobacka, T. Lindfords, M. McCarrick, A. Ivaska, A. Lewenstam, *Anal. Chem.* 67 (1995) 3819.
61. Y. Umezawa, P. Buhlmann, K. Umezawa, K. Tohda, S. Amemiyai, *Pure Appl. Chem*. 72 (2000) 1851.
62. A. Danet, L.L. Zamora, J.M. Calatayud, *J. Flow Injection Anal*. 15 (1998) 168.
63. M. Trojanowicz, M. Szewczynska, M. Wcislo, *Electroanal.* 15 (2003) 347.
64. G.G. Guibault, R.A. Durst, M.S. Frant, H. Freiser, E.H. Hansen, T.S.Light, E. Pungor, G.A. Rechnitz, N.M. Rice, T.J. Rohm,W. Simon, J.D.R.Thomas, *Pure Appl. Chem.* 48 (1976) 127.