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Fabrication of New Potentiometric Microsensor for Metformin Based on Modified Screen-Printed Microchip

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A new disposable potentiometric micro-senor responsive for metformin drug has been microfabricated using new developed approach. The organic membrane based sensitive layer was prepared by embedded the metformin : tetraphenyl borate ion association complex in plasticized PVC support matrix modified with carbon nanotubes (CNTs). The cocktail coating mixture of the sensitive layer was deposited on the surface of disposable plastic screen-printed microchip using new methodology recently developed. The microfabricated chip assembly was characterized according to IUPAC recommendations as a potentiometric microsensor for analysis of metformin drug. The merits offered by the developed novel disposable microchip include simple, high reliability, good credibility, long life span and low cost and rapid determination of metformin drug. The elaborated microchip was successfully utilized in the quantification of the metformin drug in some pharmaceutical formulations. Statistical calculations were performed to assess the accuracy and precision of the new microchip assembly for accurate determination of the metformin instead of the highly sophisticated expensive machines.

Keywords: Metformin; Screen printed microsensor; Disposable microchip; Potentiometric analysis

1. INTRODUCTION

Metformin hydrochloride drug mainly reduces the elevated blood glucose level in diabetic patients, but it does not increase insulin secretion. Consequently, it is used as an antidiabetic agent in the treatment of type 2 (non-insulin-dependent) diabetes mellitus to control high blood sugar. Controlling high blood sugar generally avoids nerve problems, kidney damages, sexual function problems and loss of limbs [1-10]. Moreover, it is useful in overweight subjects by suppressing appetite. The empirical

formula of metformin hydrochloride (N, N dimethyl imido dicarbonimidic diamide hydrochloride) is $C_4H_{11}N_5$.HCl and its molecular weight is 165.63 g/mol. Based on its usage on a large scale worldwide, continuous monitoring of metformin concentration in the pharmaceutical formulation and in human plasma is a critical issue for long time. Several instrumental techniques have been reported for determination of metformin drug [1-17]. These techniques include UV-Visible spectrophotometry [1-4], spectrofluorimetric methods [5], electrochemical methods of analysis [6-9], potentiometric microsensor [10], high performance liquid chromatography [11,12], LC-MS/MS [13, 14] and different HPTLC techniques [15-17].

Compared to the other techniques, on the other hand, the development of thin-film micro-electrodes has recently attracted an increasing interest due to their simplicity, high sensitivity, low cost, large-scale production, and short analysis time as well as their automation and integration feasibility [18-27]. On this context, recently, great efforts have been devoted by scientists for microfabrication and characterization of the modified screen-printed micro-electrodes [28-36]. Miniaturized screen-printed planar based microelectrodes which originate from the chip microelectrodes have many advantages include large mass production, disposable, low cost, simple, fast, short response times, small, accurate, robust, inexpensive, use only small aliquots of reagents and integration and automation feasibility. In addition, these miniaturized tools offer some sort of modern technology such as the minimum sample volume and can be used for in vivo application. In this context, fabrication, characterization and applications of the screen-printed based micro-electrodes become very essential for analytical laboratories. These small devices represent very promising and cost-effective tools for reliable analysis of many species particularly drugs whether species in their pharmaceutical formulations and biological samples. Thus, there has been a growing interest in the fabrication of small size disposable, new miniaturized, planar, and all-solid-state microchips suitable for industrial, biomedical, environmental applications, particularly in the field of drug analysis. Screen printed electrodes based on thick-film of silver/silver chloride were demonstrated as reference electrodes [28, 29]. Different membrane sensitive layers were reported in the microfabrication of screen-printed microchips for some toxic metal quantifications [30-33]. All-solid-state microchips based on screen-printed electrodes prepared by different sensitive materials responsive for different biological species have been reported [34-37]. To the best of our knowledge, few publications described the fabrication of a screen-printed microchips for drug species analysis have been appeared in the literature [24, 38, 39]. Moreover, it was reported that the potentiometric response parameters of the screen-printed based microsensors have significantly enhanced upon embedded nanoparticles material into the membrane sensitive layer. Thus, different nano-particles based sensitive materials have been used to improve the electrochemical behavior of screen printed electrodes [33, 40-42].

Based on the above-mentioned studies, miniaturization of the instrumental analytical techniques become very interesting goal due to their huge number of advantages. Realization of modified screen-printed based microchips as an example of such simple and low-cost devices instead of the sophisticated and expensive machines represents important achievements in the analytical laboratories. In this context, here in this work, we demonstrated for the first time the microfabrication of organic membrane based thin-film microchip assembly modified with carbon nanotubes for metformin measurements using new methodology recently developed.

2. MATERIALS AND METHODS

2.1. Materials and reagents

All the used reagents and chemicals were of analytical reagent grade, unless otherwise stated. Moreover, double distilled water produced using Aquatron water distiller (A4000D, Bibby Scientific, UK, 1.0 MΩ cm^{-1}) was used to rinse the glassware and in the preparation of the reagents, throughout. In the characterization studies, all reagents were analytical reagent grade chemicals prepared in double distilled water. In the selectivity studies, chloride or nitrate salts of the metal used were purchased from Riedel-de Haën. Screen-printed plastic micro-chips working carbon electrode (0.25 mm PET, 3 mm/6 mm in diameter, graphene modified SPE) was purchased from Suzhou Delta-biotech (Ltd, China) and used as the microelectrode substrate. Multiwall carbon nanotube purified (id: 5-12 nm, od:30-50 nm, length: 10-20 µm, purity: >95%,) was purchased from Chengdu organic chemicals Company "COCC", China. Potassium Tetrakis (4-chlorophenyl) borate lipophilic additive and solvent mediator, 2-nitrophenyl octyl ether were purchased from Sigma-Aldrich (CH-9471 Buchs, Switzerland). Tetrahydrofuran (THF) and poly (vinyl chloride) high molecular weight (220,000, PVC) carboxylated were purchased from Riedel-de Haën chemical Company (Germany). Metformin hydrochloride raw material (purity: 99.6 %) was a gift supplied by Aljazerah Industry from Auro laboratories company (India). Different formulations of metformin drug were collected from local pharmaceutical stores and used in the application studies of the microchip.

2.2. Instrumentations

The electrochemical characterization measurements were carried out at room temperature, using Jenway (model 3510) pH/mV meter and Jenway combination pH electrode for all pH experiments. An assembly of metformin : tetraphenyl borate ion pair complex modified with carbon nanotube based screen-printed microchip was used for all potentiometric measurements as working electrode responsive for metformin in conjunction with a Metrohm double junction reference electrode.

2.3. Microfabrication and electrochemical evaluation of the screen-printed metformin microchip

In our previous work, the microfabrication of plastic screen-printed electrode integrated with organic membrane sensitive layer was reported for the first time using cost effective, fast and simple new approach [27]. In this technique, plastic disposable screen-printed microchip was rinsed in double-distilled water and left to dry in air prior being used as electrode substrate in all assemblies (Fig. 1). Two assemblies of the micro-sensor (microchips 1 and 2,) containing different ratio of the nano-composite sensitive materials, as summarized in Table 1, were fabricated and investigated as metformin potentiometric microchips. The cocktail coating mixture was prepared, for each assembly, by thoroughly mixing anion excluder potassium tetrakis (4-chlorophenyl) borate, the ionophore (plasticized metformin : tetraphenyl borate ion pair complex, carbon nanotubes composite), poly (vinyl chloride) support in tetrahydrofuran (THF) as a solvent in small beaker. The sensitive layer mixture, was then transferred

into homemade manual small nebulizer and sonicated for 2 h. prior being used as a sensitive membrane coat. Prior the deposition of the sensitive layer, the metal contacts of the screen-printed microchip substrate were tightly sealed using tissue paper. Aliquots of few micro-liter of the organic membrane sensitive layer were nebulized successively, onto the surface of screen printed microchip for few seconds, in fume hood. After each successive nebulization step, the very thin layer deposited of the coat was then left in air for 2-3 min, for solvent volatilization. The desired uniform layer of the organic membrane sensitive coat covers the substrate surface was obtained by successive repetition of the nebulization steps several times. Prior the nebulization process, the cocktail coating sensitive mixture was sonicated for 2 h. and between the successive nebulization steps for 3 min as well, to spread out the nano-particles. Three chips were microfabricated for each assembly and used in the characterization and applications studies of metformin analysis (Fig. 1). The realized metformin microchips were soaked in 10^{-2} mol L^{-1} metformin hydrochloride solution for 1 h. before measurements and store dry in air when not in use.

Figure 1. Photographic picture of fabricated screen-printed microchip assemblies.

Table 1. Sensitive layer composition of metformin microchip assemblies.

Microchip No.	Ionophore composite, 14 mg		Anion Excluder, mg	ONPOE, mg	PVC, mg
	CNTs %	Ionophore %			
1	0	100	6	114	66
2	5	95	6	114	66

3. RESULTS AND DISCUSSION

The microfabrication of disposable plastic screen-printed microchip based on organic membrane sensitive layer (organic membrane onto plastic substrate) was realized for the first time in our previous work [33] using a new nebulization technique. Using this approach, screen-printed microchip assembly based on plasticized tetraphenyl borate : metformin ion pair complex sensitive material modified with carbon nanotubes has been realized and electrochemically characterized according to IUPAC

recommendations as metformin potentiometric micro-sensor. The combination of the organic layer sensitive membrane with the plastic screen-printed substrate microchip provides significant merits. The screen-printed microchips have the advantages of simple construction, small size, cheap, mass production and have versatile applications, while the organic layers sensitive membrane was widely used in many chemical electrodes due to their simplicity, high selectivity and sensitivity. Based on the merits offered by this combination, metformin screen-printed microchip has been fabricated which belongs to a new generation of screen printed microchips realized using this new approach. The new methodology dramatically modifies the realization of a huge number of organic membrane based screen-printed microchips responsive for drugs, biological species, toxic elements, pollutants and heavy metals. The miniaturized small sized devices will improve the integration and automation feasibility and therefore, enhanced such microchips application in sensors network.

3.1. Electrochemical evaluation of the metformin microchip

Two assemblies (Fig.1) of the organic membrane based screen-printed microchips having the same content of anion excluder, solvent mediator and PVC support but with different composition of the sensitive element (Table 1) have been fabricated (three copies for each one) and potentiometrically evaluated as metformin microchips according to IUPAC recommendations. Triplicates potentiometric calibration responses of the metformin microchip based solely on the tetraphenyl borate : metformin ion pair complex as ionophore without carbon nanotubes (CNTs) (assembly number 1) have been presented in Fig. 2. The chips based on this composition offered sub Nernstian response of potentiometric sensitivity of (47 ± 3) mV / concentration decade. However, the potentiometric response of the metformin microchip significantly enhanced upon addition of (CNTs) to the sensitive element (5 % CNTs, 95 % tetraphenyl borate : metformin ion pair complex ionophore, assembly number 2). The chips based on this assembly offered super Nernstian response towards metformin with a sensitivity of 61±1 mV/concentration decade. Triplicates potentiometric calibration responses of the CNTs / ionophore composite based microchips were presented in Fig. 3. This microchip assembly, therefore, has been selected for the rest of characterization and application studies. The dramatic enhancement in the sensitivity upon addition of CNTs is attributed to the unique and interesting properties of such nano-structured species induced by the high surface to volume ratio. These nano-based materials offered superior ion-exchange properties and excellent electrical conductivity [27, 33]. However, it was reported in our previous work that increasing CNTs content in the composition of the sensitive membrane (more than 5 %) gradually decreases the chip sensitivity towards the measured species due to the decreasing of the ionophore percentage [33].

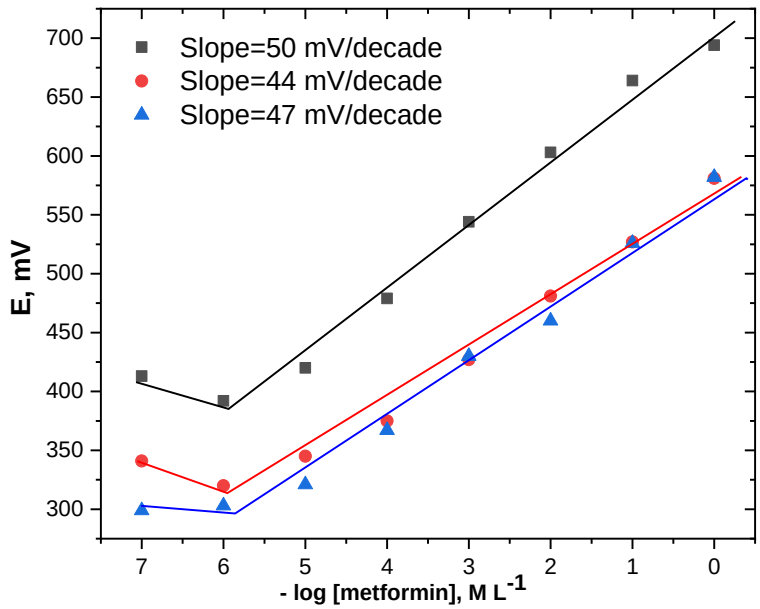

Figure 2. Triplicates potentiometric calibration responses of metformin microchip 1(assembly without CNTs).

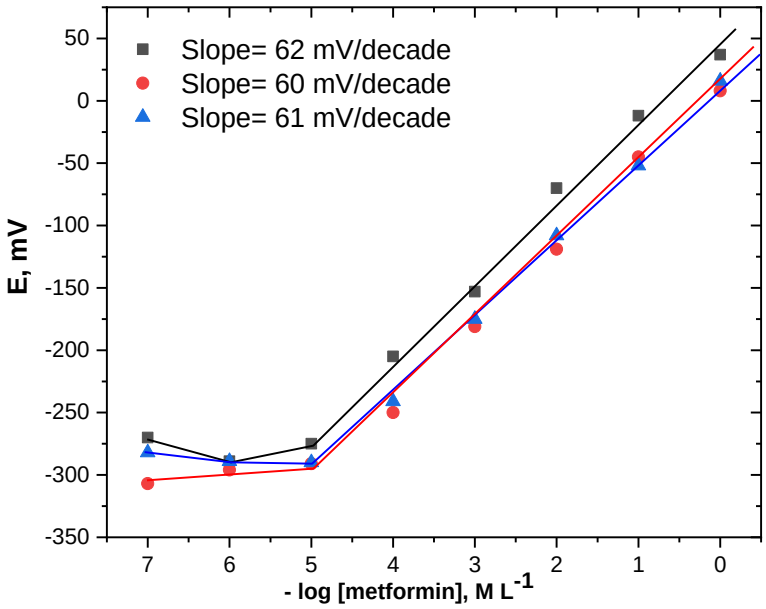

Figure 3. Triplicates potentiometric calibration responses of metformin microchip 2 (assembly with 5% CNTs).

The potentiometric dynamic response time of the metformin chip assembly has been tested by successive monitoring of the time required to obtain the constant potential after dipping the microchip in a series of 10^{-5}-10^{-1} mol L^{-1} of metformin solutions each having tenfold increase in concentration. The data obtained presented in Fig. 4 showed that the metformin microchip based on assembly number 2

offered a very fast response time (10 s) in the investigated linear range. In addition, the long-life span of the realized metformin microchip has been assessed by performing frequent calibration of the microchip using freshly prepared metformin solution. The results obtained showed that, the elaborated microchip provides a relatively long-life time (> 8 months).

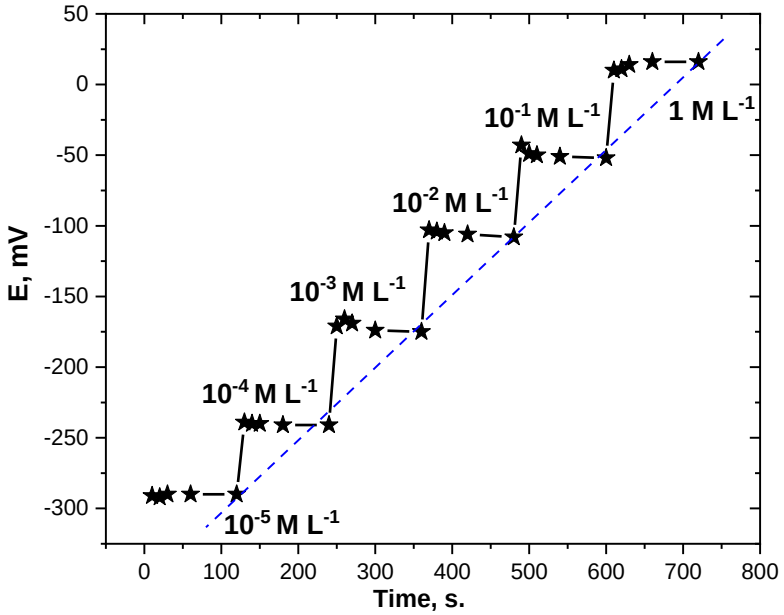

Figure 4. Potentiometric dynamic response of metformin microchip 2 (assembly with 5% CNTs).

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On the other hand, two different solutions of metformin (10^{-2}, 10^{-3} mole L^{-1}) have been used to study the influence of the pH of the test solution on the potentiometric response of the realized metformin microchip assembly. In this study, the pH of the test solutions has been changed by the addition of small aliquots of diluted sodium hydroxide and nitric acid. The change in potential of the elaborated metformin microchip (E, mV) is recorded versus the pH of the test solutions and the results obtained has presented in Fig. 5. As can be seen, the potential of the metformin microchips is almost not affected by the changes in pH of the test solution in the range of 4.0 – 10.9. Consequently, this pH range of the test solution represents the pH applicability of the elaborated metformin microchip. Therefore, the characterization and application studies have been performed in this pH range. The obtained lower potential at higher acidic and basic media probably due to change of the membrane phase and deterioration of the membrane coat, respectively.

To assess the ability of the elaborated microchip to quantify the metformin ion in the presence of some interferants ions, the potentiometric selectivity coefficient has measured using separate solution method (SSM). The data obtained summarized in Table (2) showed that the metformin microchip assembly offers reasonable selectivity towards primary ion with respect to the investigated monovalent, divalent and trivalent cations. For comparison, the electrochemical response properties of the two different assemblies of the elaborated microchip are summarized in Table (3).

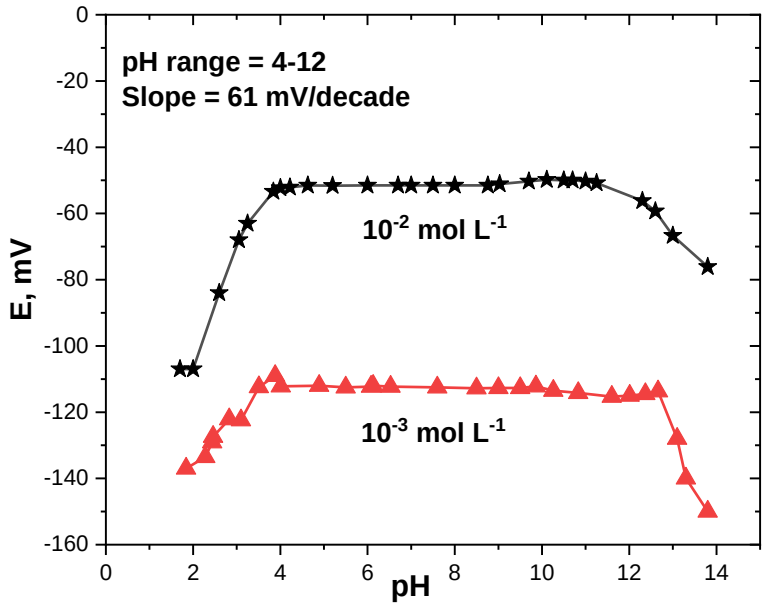

Figure 5. Effect of pH on the potentiometric response of metformin microchip 2 (assembly with 5% CNTs).

As can be seen the metformin microchip based on the assembly number (2) containing CNTs 5% of the sensitive element provides super Nernstian response with sensitivity of 61±1 mV/concentration decade covering the linear range of metformin concentration of 10^{-5}–1 mol L^{-1} with a detection limit of 4×10^{-6} mol L^{-1}, fast response time (< 10 s.) and relatively long-life span (> 8 months). These electrochemical response parameters of the elaborated metformin microchip are almost the same and in some cases more better than those of the electrochemical sensor previously reported for metformin quantifications [1-3, 5, 6]. Moreover, the development of miniaturized metformin microchips offers the merits of small size, simple microfabrication, fast response time, mass production automation and integration feasibility.

Table 2. Selectivity coefficient ($K_{i,j}^{pot}$) of metformin microchip (10^{-3} mol L^{-1}).

Interfering ion	$K_{i,j}^{pot}$
Metformin	1
Na^{+}	7.9×10^{-3}
Li^{+}	5.9×10^{-3}
NH_4^{+}	5.1×10^{-3}
Mg^{2+}	7.7×10^{-3}
Ca^{2+}	1.3×10^{-2}
Cu^{2+}	1.6×10^{-3}
Cd^{2+}	2.0×10^{-3}
Fe^{3+}	1.3×10^{-2}
Cr^{3+}	1.9×10^{-3}

Table 3. Response parameters of the metformin microchip assemblies.

Parameter	Microchip 1	Microchip 2
Slope, mV/decade	47±3	61±1
Linear range, mol L^{-1}	10^{-6}–1	10^{-5}–1
Detection limit, mol L^{-1}	10^{-6}	4×10^{-6}
Response time, s.	< 10	< 10
Life span, months	> 8	> 8
pH range	–	4.0–10.9

3.2. Analytical applications of the metformin microchip

To investigate the credibility and reliability of the elaborated screen-printed based microchip assembly for quantification of metformin from real samples, the chip assembly has successfully applied for the quantification of metformin in some real samples of different drug formulations. In this study, different drug formulations of metformin hydrochloride have been collected from the local pharmaceutical stores in Saudi Arabia and analyzed using the realized microchip.

Table 4. Determined pharmaceutical formulations of metformin hydrochloride.

No.	Formulation, mg	Origin	Nominated Value, mg	*Weight of Tablet, gm
1	Tablet, 500	Oman	500	0.602
2	Tablet, 750	Saudi Arabia	750	1.093
3	Tablet, 850	France	850	0.897
4	Tablet, 1000	France	1000	1.071

**Mean value of the weights of three tablets.*

Table 5. Determination of metformin in different formulations using the microchip.

No.	Formulation, mg	Added (nominated) Value, mg	Measured Value, mg	Recovery, %
1	Tablet, 500	83.0	74.5	89.7
2	Tablet, 750	68.0	68.5	100.7
3	Tablet, 850	94.0	91.1	96.9
4	Tablet, 1000	93.4	88.6	94.8
Average recovery				95.5

The nominated values, origin of the drug and the weight of each tablet of the investigated drug formulations were summarized in Table (4). In this study, three tablets containing metformin hydrochloride of each investigated formulation were weight and finely powdered. Accurately weight portion of 100 mg of the metformin hydrochloride powder was dissolved in 100 mL of deionized water, mixed for about 15 min, sonicated for 30 min in a sonicator. These real samples of the different drug

formulations have been quantified by the elaborated metformin chip assembly using the potentiometric calibration plot performed at the same conditions. The results obtained were summarized in Table 5. As can be seen, the concentration values determined using the realized micro-chip offers a satisfactory good agreement with the nominated values of the different tested formulation with average recovery of 95.5 %.

4. CONCLUSION

Fabrication, potentiometric evaluation and analytical applications of new screen-printed microchip assembly based on tetraphenyl borate : metformin ion pair complex ionophore modified with 5 % CNTs responsive for metformin drug have been demonstrated in this work. Using novel nebulization technique recently developed, the metformin based disposable chip assembly has been realized for the first time. The microfabricated metformin chip assembly offers high sensitivity (61±1 mV/decade), good selectivity, short response time (< 10 s.), long life span (> 8 months) and automation and integration feasibility. The disposable microchip assembly has been successfully used in the quantification of metformin in some real samples of different drug formulations with average accuracy of 95.5 %.

CONFILCT OF INTEREST
The authors declare that there are no conflicts of interest with regards to this work.

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