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Development of New Thin-Film Micro-sensor for Potentiometric Determination of Amiloride

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A new simple, selective, rapid and precise thin-film organic membrane based micro-sensor was developed for potentiometric determination of amiloride drug. The membrane based microchips was constructed using (amiloride:tetraphenyl borate) ion pair as sensing material, o-nitophenyloctyl ether as plasticizer, potassium tetrakis(4-chlorophenyl) borate as anions excluder and PVC as supporting matrix. The thin-film micro-sensor based on this composition provides linear Nernestian response with sensitivity of 60±1 mV/ concentration decade covering relatively wide concentration range of 5×10^{-6} - 1×10^{-2} mol L^{-1} of amiloride over pH range of 3-8. The suggested micro-sensor provides fast response time (<10 s) and good selectivity for amiloride over some tested cations. The microchip was successfully applied in direct potentiometric determination of amiloride in its pharmaceutical formulation. The average recovery was 96% with a mean standard deviation <4%.

Keywords: Amiloride, thin-film micro-sensor, organic membrane, potentiometric determination, pharmaceutical formulation**.**

1. INTRODUCTION

Amiloride HCl drug designated chemically as 3,5-diamino-6-chloro- N-(diaminomethylene) pyrazinecarboxamide monohydrochloride, dehydrate. It has empirical formula of $C_6H_8ClN_7O.HCl.2H_2O$ and its molecular weight is 302.12. It used as antikaliuretic-diuretic agent for people with high blood pressure or fluid retention due to congestive heart failure. Amiloride HCl is a potassium-conserving (antikaliuretic) drug that possesses weak natriuretic, diuretic, and antihypertensive activity [1-11]. Literature survey reveals that several analytical methods have been

reported for the determination of amiloride HCl drug alone or in combination with other drugs including spectrophotometry [1-6], chromatography [1, 7-10], chemometry [11] and voltametry [12]. These methods are expensive and require sophisticated machines, tedious procedures and frequent calibration. Few ion selective electrodes have been reported for determination of amiloride, although such devices offers several advantages such as simple instrumentation, ease of preparation and procedures, reasonable selectivity, relatively fast response, wide dynamic range, and low cost [13,14].

On the other hand, micro-sensors originate from chemical sensors have the advantageous of reduced size, small sample volume and the integration and automation feasibility [15-18]. The development of microchips based potentiometric sensors play an important role in pharmaceutical analysis due to their simplicity, rapidity and accuracy over some other analytical methods. Moreover, micro-sensors generally incorporate some sort of modern technology such as the minimum sample volume and can be used in vivo application. The advantages brought by these new microchips based sensors are; small and inexpensive; mass-produced, accurate and robust, use only small amount of reagents and short response times. Therefore, there is a quiet revolution in the developments of potentiometric sensors and microchips based sensors in the analysis of pharmaceutically active compounds.

On this context, we have successfully used a different strategies and approaches in the micro-fabrication of novel microchip based sensors [15-18]. Recently, we have developed new approach (Arida Approach) in the micro-fabrications of organic membrane based thin-film micro-sensors. The new techniques based on electrochemical treatment of the gold or platinum substrates surface in combination with nebulization of the cocktail coating mixture as a sensing material. These new techniques reveal long-live organic membrane based thin film sensors with excellent stability, reproducibility and accuracy.

Based on our knowledge, there is no any micro-sensor reported for the determination of amiloride. Owing to the importance of amiloride drug and its applications, an attempt was made in this work to develop a new organic-membrane based thin-film micro-sensor responsive for amiloride drug. The suggested microchip based sensor was applied in the determination of amiloride in some pharmaceutical formulations.

2. EXPERIMENTAL

2.1. Instrumentation

The potentiometric measurements were made at 25±1 ^{0}C, using Jenway (model 3510) pH/mV meter and Jenway combination pH electrode for all pH measurements. Amiloride:tetraphenyl borate based thin-film microchip was used for all potentiometric measurements in conjunction with a double junction reference electrode (Metrohom) containing KNO_3 (10% w/v) in the outer compartment.

The surface morphology studies of the thin-film substrate were performed using atomic force microscope, AFM (Edge Dimension, Bruker, USA) taping mode -supplied with a nanoscope drive V8.02 software- with a scan size 50×50 μm and a scan rate at 100 μm s^{-1}.

2.2. Reagents and materials

High molecular weight (220,000) poly(vinyl chloride) carboxylated (PVC) and tetrahydrofuran (THF) were purchased from Riedel-de Haën chemical company (Germany). The solvent mediator 2-nitrophenyl octyl ether, tetra phenyl borate (TPB) and the lipophylic additive potassium tetrakis(4-chlorophenyl) borate were purchased from Sigma-Aldrich (CH-9471 Buchs, Switzerland). The drug formulation of amiloride was purchased from local drug stores. A gold thin-film silicon-planner microchip was used as the substrate for the sensor assembly. All other chemicals were of analytical regent grade and deionized water with a Milli-Q water purification system (Millipore, 18.3 MΩ cm^{-1}) was used throughout the experiments. A stock solution of amiloride 1×10^{-2} mol/L was prepared and used in the preparation of freshly diluted amiloride solutions ($1x10^{-3}$-$1x10^{-7}$ mol/L) which then used in the calibration of the suggested thin-film micro-sensor.

2.3. Fabrication and evaluation of amiloride microchip

The ion pair complex, amiloride:tetraphenylborate sensitive material was prepared by mixing aliquots of 15 ml of 10^{-2} mol/L amiloride solution with 15 ml of 10^{-2} mol/L sodium tetraphenylborate solution. The mixture was stirred for 15 min., the obtained yellowish white precipitate electroactive ion pair was filtered off, washed with deionized water and then dried overnight at room temperature.

The cocktail coating mixture was prepared by thoroughly mixing 14 mg of amiloride:tetraphenylborate ion pair sensitive material, 6 mg of potassium tetrakis (4-chlorophenyl) borate, 114 mg of 2-nitrophenyl octyl ether and 66 mg of PVC in 6 mL THF. This mixture was used as organic membrane sensitive layer.

Prior to the deposition of the organic membrane sensitive layer, the gold thin-film microchip substrates were electrochemically treated by transferred to the deposition cell containing 10^{-3}mol L^{-1} $AgNo_3$ to deposit a thin-layer from Ag precipitate as described in our previous work [15]. The proposed amiloride microchip was realized by nebulization of the cocktail coating mixture on the treated substrate surface as described elsewhere [15]. After fabrication, the micro-sensor was then air dried at ambient temperature for 24 h and used as a sensitive micro-sensor in the potentiometric measurements of amiloride. The micro-sensor was conditioned by soaking in 10^{-3} mol/L amiloride solution for 2 h before use and stored in air at ambient temperature when not in use.

All potentiometric measurements were performed using the fabricated amiloride based microchip as working electrode in conjunction with a commercial Ag/AgCl reference electrode immersed in stirred test solutions. Both the selectivity coefficient $K_{A,B}^{pot}$ (obtained by separate solution method) and response characteristics of the thin-film amiloride have been measured according to IUPAC recommendations. The elaborated microchip was calibrated by measuring the cell potential values after stabilization to ±0.5 mV in a series of amiloride solutions covering the concentration range 10^{-2}- 10^{-7} mol/L amiloride. The obtained calibration plot was used for subsequent determination of the unknown amiloride samples.

3. RESULTS AND DISCUSSION

3.1. Characterization of the thin-film amiloride microchip

The amiloride:tetraphenylborate ion pair based thin-film microchip was realized, characterized and examined as new micro-sensor responsive for amiloride drug. Prior to the potentiometric characterization of the proposed microchip, the surface morphology studies of the thin-film substrate before treatment, after treatment and after applying the organic membrane sensitive layer were conducted using atomic force microscope (AFM). The results obtained were presented in fig. 1. The high resolution of AFM which far exceeding that of optical methods makes it superior in studying surface roughness (Ra). As can be seen, the untreated surface of the substrate (*a*) exhibits very smooth surface with very low roughness. The roughness which is the difference between the highest and lowest points on the surface (Ra) is 0.182 V. After electrochemical treatment of the substrate surface (*b*), the roughness dramatically enhanced with Ra of 0.894 V. This improved the adhesive properties of the thin-film substrate, decreased the peeling of the membrane sensitive layer from the substrate and consequently increased the stability and life span of the micro-sensor. The organic membrane surface (*c*) exhibits a uniform and a texture like surface with moderate roughness between the untreated and treated surface, Ra is 0.196.

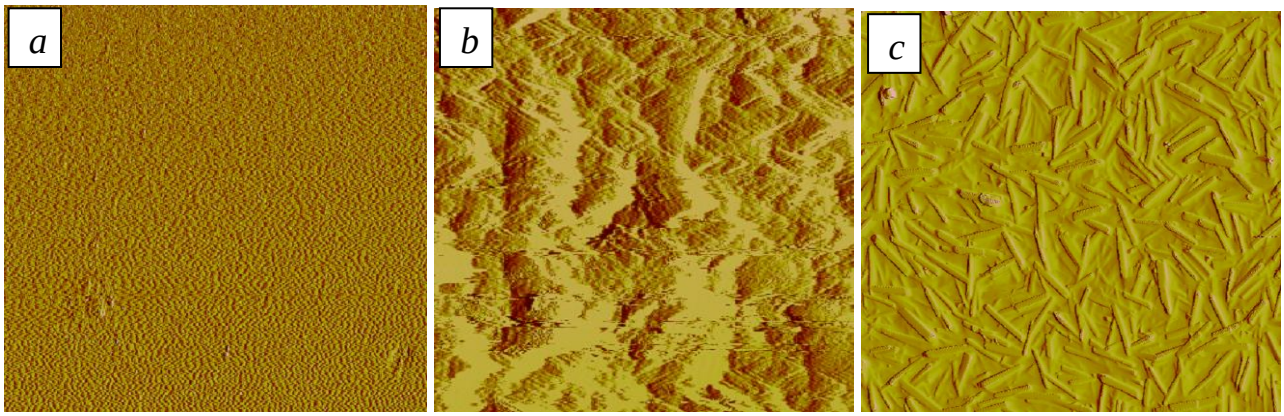

Figure 1. Typical AFM images of the gold substrate surface; (*a*) before treatment, (*b*) after treatment and (*c*) after applying the organic membrane sensitive layer.

The electrochemical response parameters of the realized thin-film amiloride micro-sensor were evaluated according to IUPAC recommendations using the micro-fabricated assembly as a working electrode. The data obtained are summarized in table 1. The results showed that the micro-sensor provides rapid, stable and linear response in the amiloride concentration range 5×10^{-6}-1×10^{-2} mol L^{-1}. The sensitivity of the calibration graph (Fig. 2) is Nernestian response with slope of 60±1 mV/concentration decade. The long term stability of the potential reading for the amiloride micro-sensor is within ±5 mV during the lifetime (4 months). Further, the responses displayed by the proposed micro-sensor for the same solutions-in the linear concentration range of amiloride- in the same day do not vary by more than ±2 mV (n=3). The lower limit of detection determined from the

intercept of the two lines of the calibration graph is 3.98×10^{-6} mol L^{-1}.

Table 1. Electrochemical behavior of the thin-film amiloride micro-sensor.

Parameter	Response
Slope (mV/decade)	60±1
Linear range, (mol L^{-1})	5×10^{-6} -1×10^{-2}
Lower limit of detection, mol L^{-1}	3.98×10^{-6}
Response time, s	<10
Life time (month)	>4
Working pH range	3-8

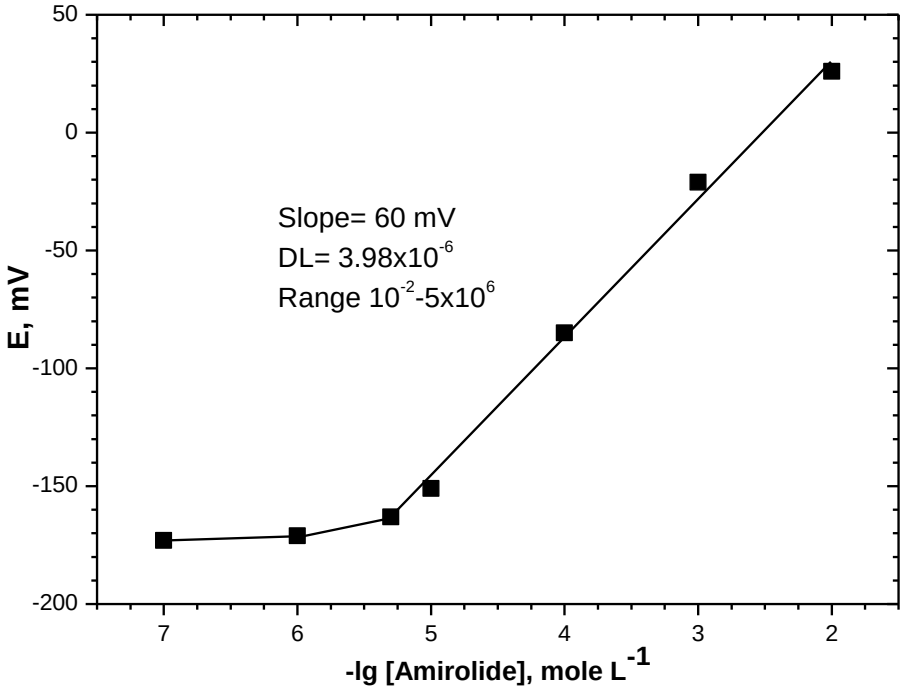

Figure 2. Potentiometric calibration response of amirolide thin-film micro-sensor.

The reproducibility of the potential reading, calibration results and sensitivity of the elaborated amiloride micro-sensor was investigated. The results obtained presented in figure 3 showed that, the calibration data from day to day were reproducible and have no significant differences in the potential reading, linear range, detection limits and sensitivity. These repeatability of the micro-sensor behavior is attributed to the strong stability of the membranes sensitive layer deposited on the treated substrate surface.

The response time of the amiloride micro-sensor defined as a length of time at which the potential reading has reach to 95% of the final potential was investigated. The results obtained presented in fig. 4 showed that, the micro-sensor provides fast response time (<10 s) in the linear concentration range of amiloride.

Based on the mentioned response parameters, the proposed amiloride micro-sensor offer electrochemical characteristics comparable -and better in some parameter- with those reported for the bulk amioride electrodes [13,14]. Further, the elaborated microchip has the advantages of reduced size, small sample volume, integration and automation feasibility.

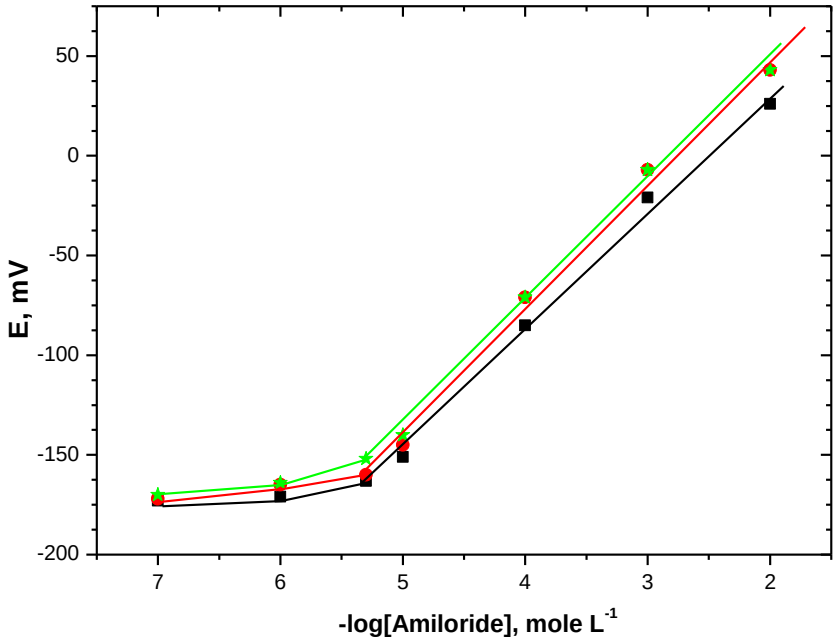

Figure 3. Triplicates potentiometric response of amiloride thin-film micro-sensor.

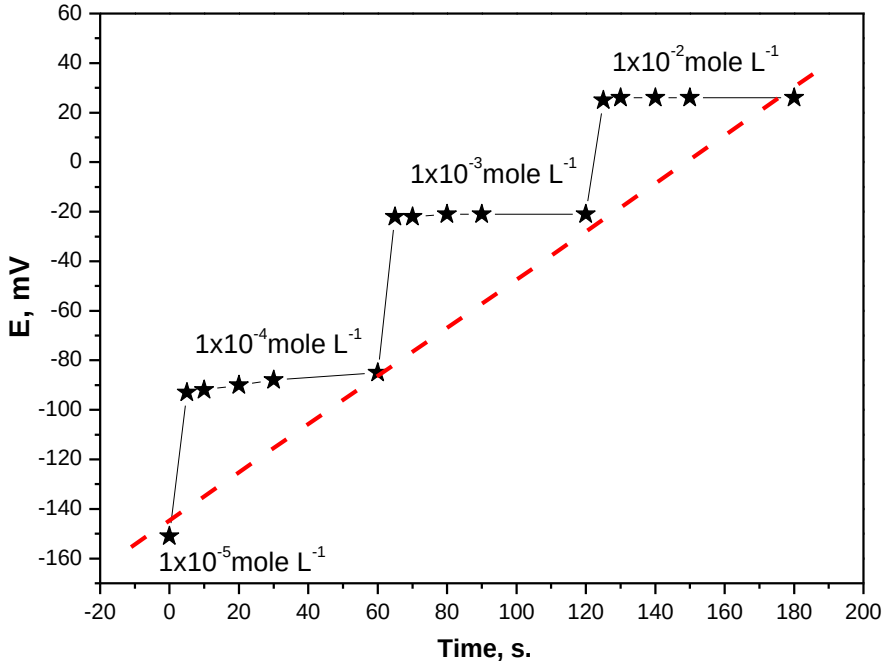

Figure 4. Potentiometric dynamic response of amirolide thin-film micro-sensor.

The potentiometric response of the amiloride thin-film micro-senor in the presence of some tested interferent monovalent, divalent and trivalent ions was tested by the separate solution method. The selectivity coefficients values $K_{A,B}^{Pot}$, are summarized in table (2). The results indicated that the suggested micro-sensor provides a reasonably selectivity for amiloride over most of the tested species.

Table 2. Selectivity coefficients of amiloride microchip.

Interferent ion	$K_{A,B}^{pot}$	Interferent ion	$K_{A,B}^{pot}$
AM-HCl	1	Mg^{2+}	2.09×10^{-4}
K^+	2.24×10^{-2}	Cu^{2+}	2.24×10^{-4}
Li^+	1.85×10^{-3}	Pb^{2+}	2.51×10^{-3}
Na^+	4.30×10^{-3}	Cd^{2+}	3.16×10^{-4}
NH_4^+	1.52×10^{-2}	Fe^{3+}	2.07×10^{-4}
Ca^{2+}	2.09×10^{-4}	Cr^{3+}	2.71×10^{-4}

The influence of pH of the test solution on the performance of the elaborated amiloride micro-sensor has been investigated using solutions of 1×10^{-3} and 1×10^{-2} mol L^{-1} of amiloride. This study was achieved by the addition of very small aliquots of concentrated nitric acid and/or sodium hydroxide solutions to the test solution. The results obtained are plotted in figure 5. As can be seen, the micro-sensor response are insensitive to pH changes in the rang 3-8. At higher pH values, free base precipitates in the test solution was obtained which result in decreasing the micro-sensor potential response.

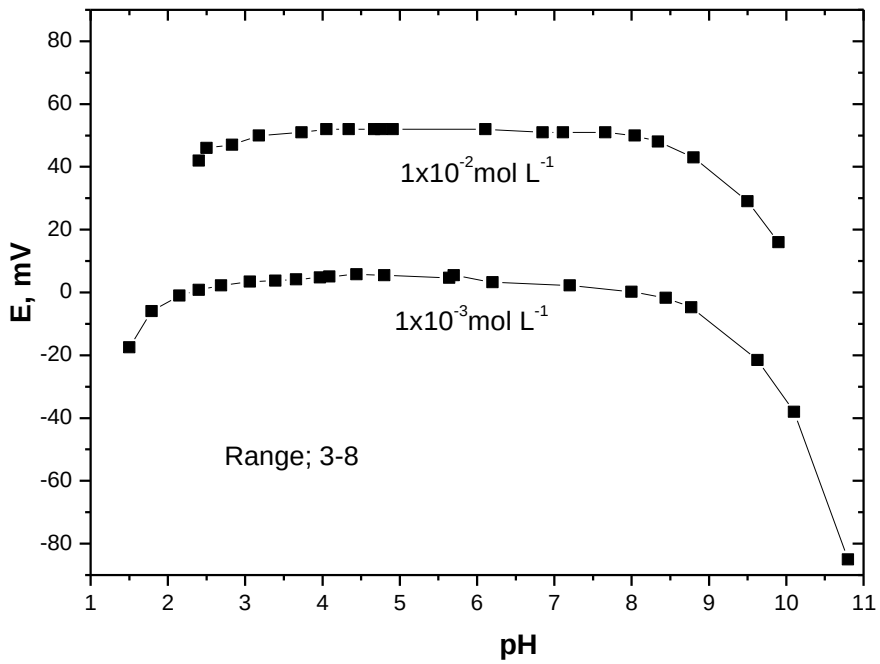

Figure 5. Effect of pH on the potentiometric response of amiloride micro-sensor.

3.2. Analytical application of amiloride thin-film micro-sensor

In order to evaluate the reliability and credibility of the elaborated amiloride micro-sensor, it was successfully used in the quantification of the drug in some pharmaceutical formulations. The drug was determined in some aqueous solutions of different concentrations of amiloride by direct potentiometry using the calibration graph obtained from micro-fabricated assembly. The data obtained are summarized in table 3. The results revealed that, the micro-fabricated assembly provides reliable, credible and analytically useful response characteristics in the quantification of amiloride in the tested pharmaceutical formulations with high accuracy (the average recovery is 96.0%) and good precision (the mean standard deviation is <4%).

Table 3. Amiloride assay using the elaborated microchip, (n=5).

No.	Taken, mole L^{-1}	Micro-sensor	
		Found, mole L^{-1}	Recovery, %
1	$1.0x10^{-5}$	$0.91\pm0.12x10^{-5}$	91.0
2	$1.0x10^{-4}$	$1.0\pm0.2x10^{-4}$	100.0
3	$1.0x10^{-3}$	$0.97\pm0.3x10^{-3}$	97.0
Average			96.0

4. CONCLUSIONS

Micro-fabrication, characterization and analytical application of novel amiloride microchip have been demonstrated. The thin-film micro-sensor provides fast and linear Nernestian response over a wide range of amiloride concentration. The micro-sensor has been successfully used in the determination of amioride content in some pharmaceutical formulations with high accuracy and precession. The assembly present simple, low cost and selective method for direct determination of amiloride in aqueous media without prior separation.

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