New Electrochemical probe for Determination of Amiloride in Pure Form and in Biological and Pharmaceutical Formulations

Research · September 2015

DOI: 10.13140/RG.2.1.3043.1204

CITATIONS
0

READS
42

1 author:

Shamah Ghorzan
Najran University
1 PUBLICATION **0** CITATIONS

SEE PROFILE

New Electrochemical probe for Determination of Amiloride in Pure Form and in Biological and Pharmaceutical Formulations

R. A. Farghali[1,3] *, Rasha G. Ahmed*[2,3]*, *Shamah Nasser Hussain Ghurzan*[4]

1 Chemistry Department, Faculty of Science, Cairo University, Giza 12613, Egypt.

2 Chemistry Department, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt.

3'4 Taif University, Faculty of Science, Chemistry Department, Taif, Hawiya 888, Saudi Arabia
*E-mail: drrasha_73@yahoo.com

ABSTRACT

A rapid and simple method was developed for Amiloride hydrochloride drug determination by electrochemical analysis using Poly(3, 4-ethylene-dioxythiophene) (PEDOT) modified glassy carbon electrode (GCE). Initial investigations were undertaken using cyclic voltammetry (CV),and differential pulse voltammetry (DPV); by scanning the potential from 0.00 to +1.80 V employing a scan rate of 50 mV/s; in the presence and absence of Poly(3, 4-ethylene-dioxythiophene) (PEDOT) to verify the voltammetric behavior of Amiloride in different media. The presence of (PEDOT) coat on the surface of (GCE) plays a key role in enhancing the current signals for the drug and thus the sensitivity of the method. Cyclic voltammetry (CV) study indicates that the oxidation process is irreversible. Parameters affecting on this method are optimized, under optimal condition The sensor showed a good reproducibility, high stability, sensitivity and anti-interference ability, and thus the sensor was further utilized to determine Amiloride level in human urin as well as some of its drug formulation and satisfactory results are obtained with law limit of detection was found to be 5.5×10^{-8} $molL^{-1}$. An interference study was also carried in presence of high concentration of ascorbic acid (AA) and uric acid (UA) to estimate the high selectivity of the electrode.

Keywords: Amiloride hydrochloride (AMICL), Poly(3, 4-ethylene-dioxythiophene) (PEDOT), Glassy carbon electrode (GCE), cyclic voltammetry (CV) , and differential pulse voltammetry(DPV).

1. INTRODUCTION

Amiloride hydrochloride, (AmilCl), N-amidino-3,5-diamino-6-chloropyrazine-2-carboxamide hydrochloride (Scheme I), is a weak diuretic that acts mainly on the distal renal tubules. It is described as potassium sparing, it increase the excretion of sodium and reduces the excretion of potassium. It is used with other diuretics in the treatment of hypertension [1].

Fig.1. Structural formula of amiloride hydrochloride

Several analytical methods have been reported for the determination of amiloride hydrochloride, including, spectrophotometry [2-13], high performance liquid chromatography [8, 14- 24], fluorimetry [25-31] , capillary zone electrophoresis [32,33] , chemiluminometric [34], potentiometry [35,36].Most of these methods are expensive, lake of sensitivity , Other methods have been reported for electrochemical determination of AMICL which generally based on reduction of this drug have been studied [37-39],New electrochemical oxidation method of AMICl at at a modified glassy carbon electrode with PEDOT using cyclic and differential pulse voltammetry, and a procedure for the determination of the drug in its pharmaceutical formulation was optimized.

2. EXPERIMENTAL

2.1. Reagents and Materials

All chemicals used as received without further purification. Pure grade amiloride hydrochloride, dihydrate and the pharmaceutical preparation Moduretic (5 mg amiloride hydrochloride, 50 mg hydrochlorothiazide/tablet) were supplied by Kahira Pharm. & Chem. Ind. Co., Cairo, Egypt., Britton-Robinson (B-R) buffer pH 2.0-9.0[40] was prepared from a mixture of 0.12M acetic acid,0.12M orthophosphoric and 0.12Mboric acids and adjusted to the required pH with 0.2 M sodium hydroxide was prepared. (3, 4-ethylene-dioxythiophene) (EDOT), Lithium perchlorate, Acetonitrile are HPLC grade. Diluted working standard solutions were then prepared daily with deionized water just before use.

2.2. Apparatus and measurements.

Voltammetric and electrochemical measurements were performed using Metrohm Autolab electrochemical analyzer (Autolab PGSTAT302N) using

NOVA software. Three electrodes assembly cell consisted of glassy carbon electrode (GCE) as working electrode, an Ag/AgCl in 3 mol/L NaCl as a reference electrode and platinium wire was a 10 cm long/ 2.0 mm diameter as an auxiliary electrode. The working electrode was polished by a BAS-polishing kit with 0.3 and 0.05 µm alumina slurry, rinsed and then sonicated in deionized water before starting each experiment.

The pH measurement were carried out with Hanna pH 211 microprocessor pH meter

2.3. Electrosynthesis of Poly (3,4-ethyelenedioxythiophene) (PEDOT) Polymer

The electrochemical polymerization of EDOT have been carried out by cyclic voltammetric method in non-aqueous solution containing 0.01 M EDOT, and 0.1 M $LiClO_4$ as supporting electrolyte in acetonitrile, from –1000 mV to 1500 mV with scan rate of 50 mV/s for many repeated cycles. The PEDOT polymer forms a layer that entirely coats the GC working electrode surface. The thickness of this polymer coat was varied by repeating the voltammetric step for 2, 3, 4, 7 and 10 cycles in order to get the optimum polymer layer thickness for electrochemical studies of amiloride.

2.4. Electrochemical Measurements of amiloride

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) was used to record voltammograms using the modified PEDOT/GC electrode by scanning the potential from 0.00 to +1.80 V employing a scan rate of 50 mV/s. Appropriate quantities of AMI standard solution were introduced in the electrolytic cell, diluted with B-R Buffer, pH 2, and the measurements were done.

2.5. Analysis of urine and AMICl moduretic tablet

The utilization of the proposed method in real sample analysis was also investigated by direct analysis of AMICl in human urine samples as well as in its pharmaceutical formulation. For this purpose, the urine samples were diluted 10 times in B-R buffer (pH 2) to minimize any matrix effect. In 10 mL measuring flasks, three different amounts of 5×10^{-4}molL^{-1} AMICl solution were added to 2.0 mL of urine sample, diluted with B-R buffer(pH 2), poured into the electrolytic cell, and the corresponding DPVs were recorded. Regarding the pharmaceutical formulation of AMICl, 5 x 100 mg tablets of AMICl were weighed and then the average mass per tablet was determined. The tablets were carefully grounded to a fine powder, and then a quantity of homogeneous powder equivalent to 50 mg of AMICl was dissolved in 100 mL of water by sonication for 20 min, followed by mechanical shaking for about 10 min. The desired concentration of AMICl was obtained by accurate dilution with B-R buffer. The sample solution so prepared was added to the supporting electrolyte in the voltammetric cell and DPVs of the solution was recorded and the anodic peak current was evaluated. Furthermore, the electrode was applied for the recovery assessment of AMICl in the tablets by the standard addition method. In this respect, different standard concentrations of AMICl were added to the

tablets solution. All these samples were also analyzed by HPLC reference method [8] to compare the results obtained by the proposed method.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of of AMICl on Different Types of Working Electrodes

Figure. (2-A) shows that the suitability of the Pt and glassy carbon (GC) as substrates in the development of amiloride (AMI) sensor. The Pt working electrode do not show any electrochemical response, neither on the Pt bare nor after modification with PEDOT polymer. While on using a glassy carbon as working electrode, the voltammograms (Fig. 2B) shows a single totally irreversible broad anodic peak at +1.27 V vs. Ag/AgCl.

Electrochemical behavior of AMI was investigated on the bare as well as PEDOT polymer-modified surfaces. Cyclic voltammetry (CV) was used to record voltammograms by scanning the potential from +0.00 to +1.80 V employing a scan rate of 50 mV s^{-1} . The electrochemical response of amiloride could be enhanced by modifying the GC electrode through covering its surface by a layer of electropolymerized poly(3,4-ethylenedioxythiophene (PEDOT) polymer. It is found that the anodic peak current (I_p) is significantly enhanced (*almost doubled*) with no concurrent shift in peak potential (E_p = 1.27 V) after modification of glassy carbon surface with PEDOT polymer **(Fig. 2B).**

The enhancement of amiloride signal on modified electrode may be explained on the basis that the polymeric thick layer film permits a higher surface coverage and therefore increases the amount of the electroactive amiloride moiety consequently, this leads to a rapid electron transfer rate and to a higher adsorptive ability of amiloride at the PEDOT/GC electrode.

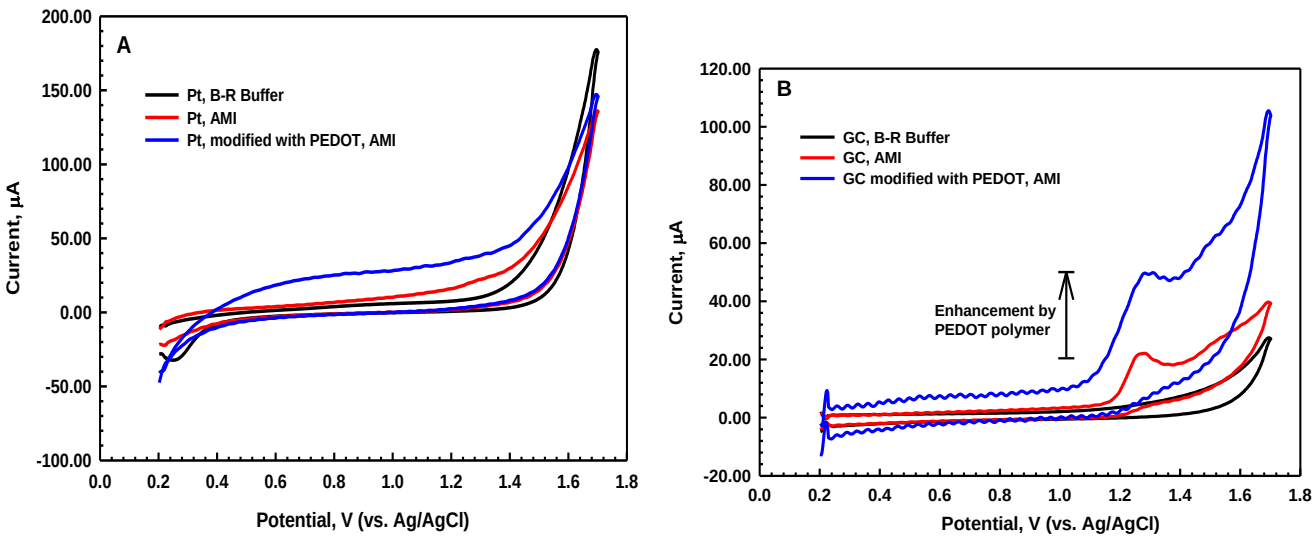

Figure 2. Cyclic voltammograms of 5.0×10^{-4} mol L^{-1} Amiloride (AMI)/ 0.1 mol L^{-1} B-R buffer, pH 2, at (A) Pt electrode, (B) Glassy carbon (GC) electrode, at scan rate of 50 mV s^{-1}.

3.2. Effect of polymer layer thickness on electrochemical response of AMICl

Figure. 3A shows that Differential pulse voltammograms (DPVs) of 5.0×10^{-4} mol L^{-1} AMI/ 0.1 mol L^{-1} B-R buffer, pH 2, at bare GC electrode and GC electrode modified with 1, 2, 3, 4, 7, and 10 polymerization cycles with PEDOT polymer at scan rate of 50 mV s^{-1} the polymeric layer obtained after two polymerization cycles outfitted the optimum thickness that give the highest possible peak current (I_p) with the lowest residual current (baseline plateau). This finding is confirmed by **figure 3B** where the number of polymerization cycles that reflects the polymer layer thickness is plotted against the electrochemical response (peak current, I_p) of *AMICl*. Therefore, two electropolymerized cycles will be the most suitable in all further studies.

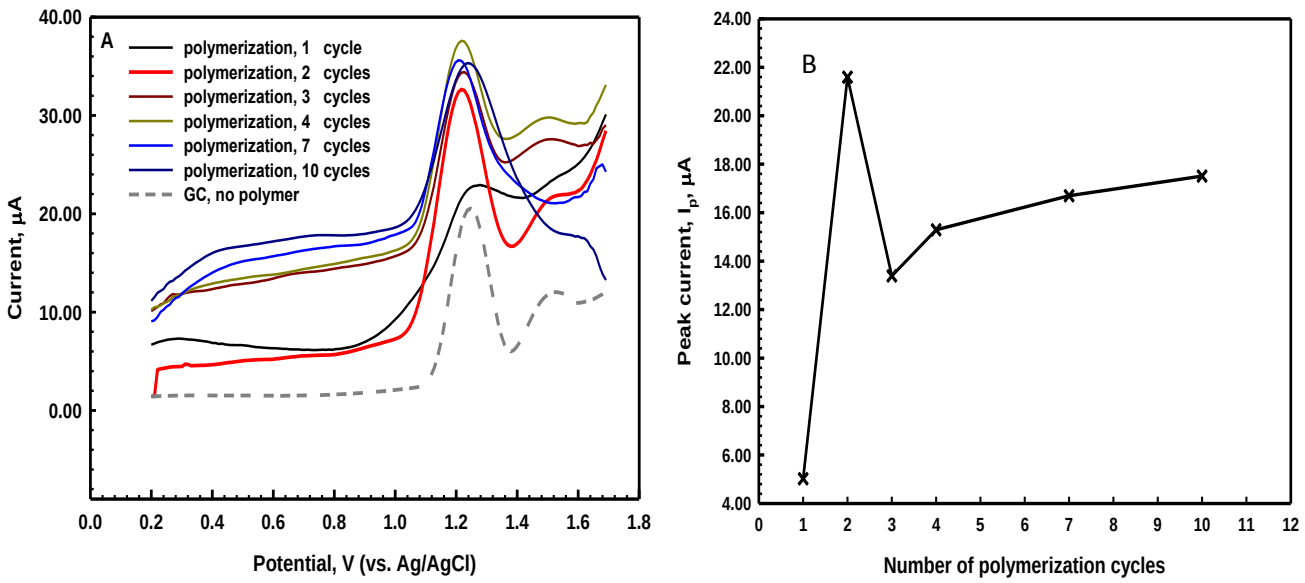

Figure 3.A) Differential pulse voltammograms (DPVs) of 5.0×10^{-4} mol L^{-1} AMI/ 0.1 mol L^{-1} B-R buffer, pH 2, at bare GC electrode and GC electrode modified with 1, 2, 3, 4, 7, and 10 polymerization cycles with PEDOT polymer at scan rate of 50 mV s^{-1}. **B)** Effect of number of polymerization cycles on electrochemical response (peak current, I_p) of AMI.

3.3. Effect of pH

The influence of pH on the voltammetric response of AMI was studied. In order to establish a suitable pH, a range of values was examined between pH 2 and 9. The voltammograms obtained for the different pH values are presented in Fig. 4A. A reasonable mechanism is presented for oxidation of *AMICl* and the pathway is shown in **Figure**.4) [41]. It can be seen that as pH of the solution increases, the anodic peak potential (E_P) shifts to less positive values. In addition, the graph of the anodic peak potential (E_P) *vs.* pH shows a good linear relation in the range of pH 2-9. The linear regression equation:

$E_p = 1.357 - 0.034\ pH$ ($R^2=0.998$) was obtained, which is indicative of equal number of electrons and protons being involved in the oxidation of *AMICl*.

The maximum current intensity (I_p) was observed for pH 2 throughout the pH range studied beyond which the peak current started to deteriorate. Thus,

B-R buffer of pH = 2 was employed for the most suitable in all further studies.

Figure .4) The Probable oxidation mechanism for AMI.

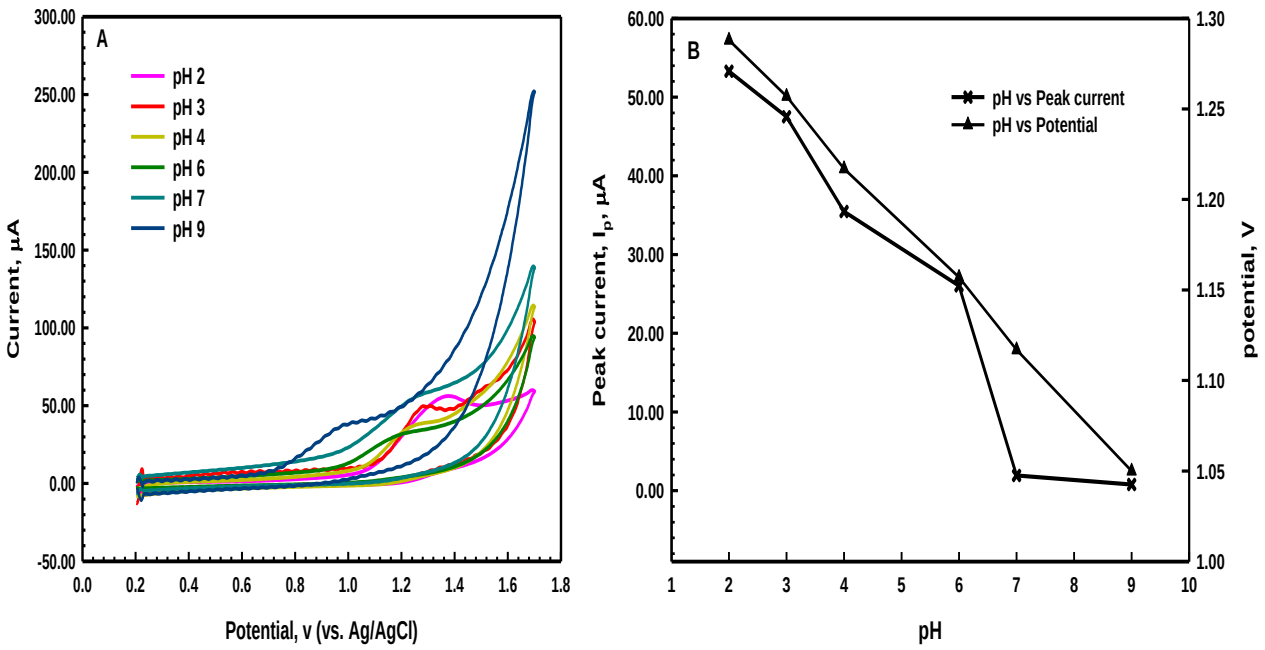

Figure 5. A) Effect of solution pH on the behavior of PEDOT/GC electrode in 5.0×10^{-4} mol L^{-1} AMI/0.1 mol L^{-1} B-R buffer: at different pH values of 2, 3, 4, 6, 7, and 9 at a scan rate of 50 mV s^{-1}. **B)** Effect of pH on peak current (I_p) (✖)

and peak potential (E_p) (▲) for 5.0×10^{-4} mol L^{-1} AMI at PEDOT/GCE, employing CV; scan rate 50 mV s^{-1}.

3.4. Effect of Scan Rate on the Voltammetric Response of AMICl

Figure .6A shows that The oxidation peak currents (Ip) of *AMICl* at PEDOT/GC (pH 2) varied with change of square root of scan rate (v) in the range of 10–130 mVs^{-1} as shown by the cyclic voltammograms. The higher the scan rate, the greater is the peak current. The logarithm of anodic peaks current were proportional to the logarithm of scan rate in the range of 10–130 mVs^{-1} (**Figure.6B**).

A linear relationship was observed between a logarithm of anodic peak current values (log Ip) as a function of logarithm of the scan rate (log ν) demonstrating that the phenomenon is diffusion-controlled with the regression equation:

LogIp (μA) =0.247 + **þ 0.603**log v (mV s^{-1}) (r^2 = 0.998)

At relatively slow voltage scans, the adsorbed layer grows much further towards the solution side and further from the electrode surface. Therefore, as the scan rate increases the flux to the electrode surface increases considerably. At relatively higher scan rates the adsorbed layer grows less further from the vicinity of the electrode.

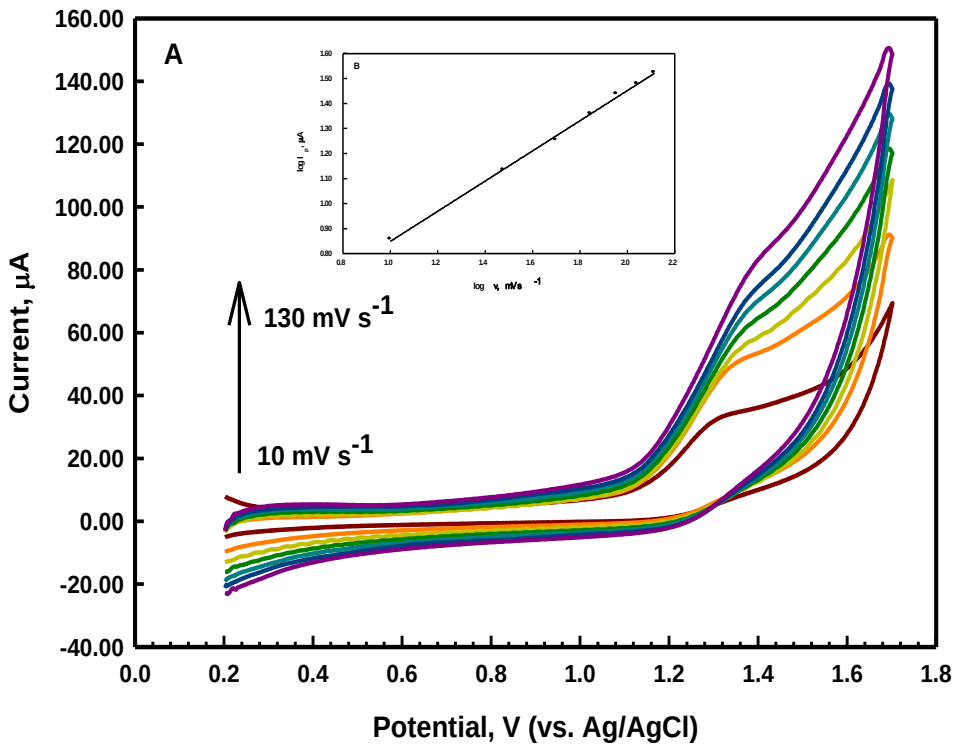

Figure .6 A) Cyclic voltammograms of 5.0×10^{-4} mol L^{-1} AMI/ 0.1 mol L^{-1} B-R buffer, pH 2, at PEDOT/GC electrode at different scan rates of 10-130 mV s^{-1} and **B)** a plot of logarithm of anodic peak current values (log I_p) as a function of logarithm of the scan rate (log ν).

3.5. Effect of Accumulation Potential and Accumulation Time

Figure.7A)shows that the effect of accumulation potential on the oxidation peak current was studied for 5.0×10^{-4} mol L^{-1} AMI/ 0.1 mol L^{-1} B-R buffer, pH 2, scan rate 50 mV s^{-1} At 20 s accumulation time. The current peak was nearly constant on charging the accumulation potential, the potential ranging from -0.6 1o +1.7 V the maximum peak current obtained at an E_a of 0.0 V used for further studies. The effect of accumulation time on the oxidation peak current was studied. increase in the accumulation time can lead to increased sensitivity of the analyte, the increase the drug concentration at the electrode surface, the effect of the varying t_a was studied for a period of 10–180 s, by maintaining E_a as a constant (at 0.0 V). It was observed that the peak current increased up to 20 s, the polymeric film has taken place at higher accumulation time (Fig. 5B). Therefore E_a of 0.0 V with t_a of 20 s were selected as the optimized parameters for the AMI determination employing DPV.

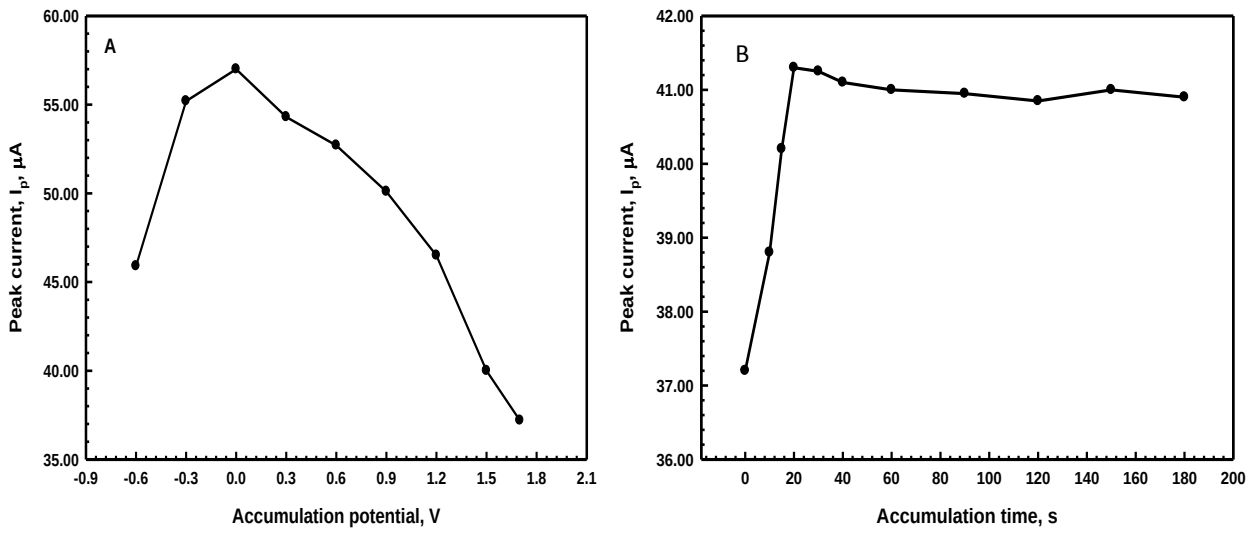

Figure 7. Effects of accumulation potential (**A**) and accumulation time (**B**) on peak current of 5.0×10^{-4} mol L^{-1} AMI/ 0.1 mol L^{-1} B-R buffer, pH 2, scan rate 50 mV s^{-1}

3.6. Interference studies: Electrochemistry of AMICl in presence of UA and AA at pH 2

The electrochemical response of AMI in the presence of AA, and UA were examined. Figure 8 shows that the DPV technique was recorded at GCE to investigate the interference study in a mixture of 1.0×10^{-3} M AA, 5.0×10^{-4} M UA and 5.0×10^{-4} M AMI as shown in figure 7.

The electrochemical oxidation of UA and AA at the modified PEDOT/GC electrode, at pH 2, occurs at approximately 0.37 V and 0.61 V, respectively, while the oxidation peak of AMI is observed at 1.21 V. These results clearly indicate that AMI shows a well-defined anodic peak that is separated from those

of UA and AA by about 0.60 V. This illustrates the good selective determination of PEDOT/GC electrode for AMI in presence of high concentration of AA and UA at pH 2.

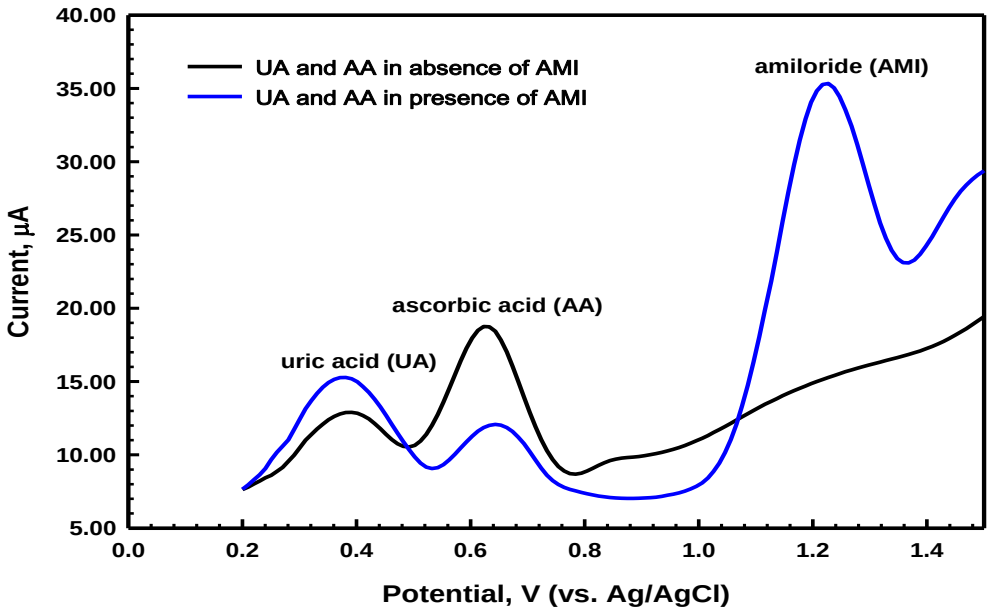

Figure 8. Differential pulse voltammograms showing the effect of interference of 1.0×10^{-3} mol L^{-1} AA, and 5.0×10^{-4} mol L^{-1} UA on electrochemical signal of 5.0×10^{-4} mol L^{-1} AMI/0.1 mol L^{-1} B-R buffer (pH 2), scan rate 50 mV s^{-1}, E_{acc} = 0.0 V, t_{aac} = 20 s, (___) in absence of AMI, (_____) in presence of AMI.

3.7. *Calibration graph, limit of detection and limit of quantitation*

Calibration curves for standard drug solution under the optimized parameters were obtained. A linear relationship was observed between 1. 0-100.0 µM AmilCl. Fig. 9 represents the differential pulse anodic voltammograms recorded using the standard addition method. The linear regression equation was

$$\mathbf{I_p\ (\mu A)\ =\ 0.278\ \mathit{C}\ (\mu M)\ -\ 0.0113} \qquad \mathbf{(R^2\ =\ 0.998)}$$

, with a correlation coefficient of 0.998, the limit of detection ($LOD = 3\sigma/b$) and limit of quantification ($LOQ = 10\sigma/b$) were calculated, where σ is the standard deviation of the intercept and b is the slope of the calibration graph. *LOD* and *LOQ* were found to be $\mathbf{1.3\times10^{-8}}$ mol L^{-1} and $\mathbf{4.3\times10^{-8}}$ mol L^{-1}, respectively. The sensitivity was calculated to be **3.92** µA μM^{-1} cm^{-2} (area of the electrode = 0.071 cm^2). , The analytical parameters for the calibration graph are summarized in Table 1.

Table 1. The analytical parameter of the calibration graph for the determination of AmilCl by differential pulse anodic stripping voltammetric method

Parameters	Values
Linear range, µg/ml	**1. 0-100.0 µM**
Slope	**0.278**
Intercept	**0.0113**
Correlation coefficient (r)	**0.998**
LOD,	**1.3×10^{-8}** mol L^{-1}
LOQ,	**4.3×10^{-8}** mol L^{-1}

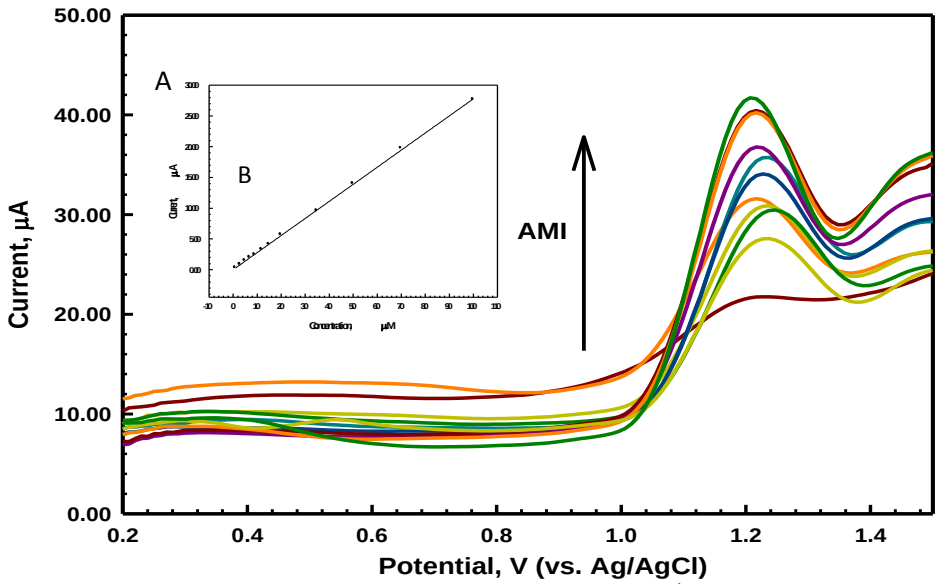

Figure 9. **A)** DPVs of 10 ml of 0.1 mol L^{-1} B-R buffer of pH 2 at PEDOT/GC electrode in different concentrations of *AMICl* (1.0 µM –100.0 µM), E_{acc} = 0.0 V, t_{aac} = 20 s. **B)** calibration curve of AMI for concentrations from 1.0 µM up to 100.0 µM).

3.8. Analytical Applications

3.8.1. Determination of AMICl in human urine

The proposed method was used to detect AMICl in urine samples, which obtained from healthy volunteer. No signal was observed for AMICl in urine samples; therefore, the urine samples were spiked by different concentrations of AMICl standard solution, and then used for further determination. The voltammograms were recorded using the DPVs, and the corresponding values were recorded. The results obtained are given in Table 2. As can be seen for the

determination of AMICl, good recoveries were obtained ranging from 96.0% and 101.7%.

Table 2. Recovery data for synthesized biological solution spiked with various amounts of 5.0×10^{-4} mol L^{-1} AMI, in fresh urine sample taken from healthy volunteers.

R.S.D. (%)[a]	Recovery (%)	Found (µmol L^{-1})	Spike (µmol L^{-1})	Urine sample
2.1	96.0	9.60	10.00	**1**
2.5	101.7	30.51	30.00	**2**
2.7	100.7	50.35	50.00	**3**

[a] Average of five replicate measurements

3.8.2. Determination of AMICl in pharmaceutical tablets

PEDOT/GCE are successfully applied for the determination of AMICl in pharmaceutical preparations by standard addition method. The amounts obtained by the proposed modified electrode are in good concurrence with the declared specifications on the pharmaceutical samples with recoveries values between 97.60 and 103.18% for five measurements. These results indicated that there is no interference from the tablet coating materials, or fillers. The results depicted in Table 3 are in good agreement with the claimed values with average recovery of 95% [42] and there is no significant difference in accuracy or precision is observed between the two methods (Table 4).

Table 3. Recovery data obtained for amiloride in pharmaceutical Moduretic tables by standard addition method.

Recovery (%)[a]	AMI found (µmol L^{-1})	AMI added (µmol L^{-1})	content (µmol L^{-1})	Sample
103.18	34.05	3.00	30.00	**1**
99.31	34.76	5.00	30.00	**2**
98.65	36.50	7.00	30.00	**3**
97.60	39.04	10.00	30.00	**4**

[a] Average of five replicate measurements

Table 4. Statistical comparison between the results of Moduretic tablets using the proposed method and the reference HPLC method.

Reference method[8]	Proposed method	Parameter
97.79	98.70	**Mean recovery, %**
1.009	2.130	**SD**
1.032	2.158	**RSD, %**
4.456		**F-ratio (6.388)[a]**
0.955		**t-test (2.780)[b]**

Average of five determinations for the proposed and reference methods.
[a] Tabulated F-value at 95% confidence level.
[b] Tabulated t-value at 95% confidence level and 4 degrees of freedom.

3.8.3. Comparison with Other Electroanalytical Cited Methods

Table 5 shows that , the response characteristics of the proposed method are compared with those obtained by some reported methods. In comparison with some other voltammetric methods for amiloride determination, our method is comparable, or even better in some aspects to those described in the literature. The designed sensor is prepared in one simple step with cheap and simple reagents and no pretreatment needed before the measurements. This gives the sensor more advantages over other modified electrodes used. This sensor showed good reproducibility, high stability, sensitivity and anti-interference ability. The sensor was further utilized to determine amiloride level in human urine and pharmaceutical formulation and satisfactory results were obtained with low detection limit.

Table 5. Comparison of the proposed method with other electroanalytical methods used for determination of amiloride.

Reference	LOD (mol L^{-1})	Linear range (mol L^{-1})	Composition of the modified electrode
[41]	7.0×10^{-9}	9.1×10^{-9}–5.1×10^{-5}	Nafion–carbon nanotube on GCE
[37]	6.3×10^{-10}	2.0×10^{-9}–2.0×10^{-7}	Dropping mercury electrode
[38]	1.0×10^{-5}	2.0×10^{-8} –1.0×10^{-6}	Dropping mercury electrode
[42]	8.6×10^{-13}	2.0×10^{-12}–1.4×10^{-11}	carbon paste electrode
[44]	5.0×10^{-7}	7.5×10^{-7}–2.4×10^{-6}	ds-DNA-modified pencil graphite electrode
[35]	9.9×10^{-7}	1.0×10^{-2}–1.0×10^{-6}	A polymeric amiloride–sodium tetraphenyl phthalate membrane electrode
[43]	1.5×10^{-6}	1.6×10^{-6}–1.0×10^{-2}	A polymeric ds-DNA –amiloride membrane electrode
This work	**1.3×10^{-8}**	**1.0×10^{-6}–1.0×10^{-4}**	**PEDOT/GC modified electrode**

4. Conclusion

This work has shown that amiloride can be determined using electrochemical method, based on Poly(3,4-ethylene-dioxythiophene) glassy carbon modified electrode, with wider linear range and lower detection limit. The proposed methods can be applied for detection of AMI in acid medium, and demonstrated that it is easily to discriminate AMI from AA and UA as common interference in biological fluids. The sensor was further utilized to determine amiloride in human urine and Moduretic tablets.

The good properties of the modified electrode, such as high sensitivity, ease of fabrication, reproducibility, and stability indicate that the proposed modified electrode will be promising for measurements of amiloride in biological fluids without any interference. This gives the sensor more advantages over other modified electrodes used in the literature. At the same time, it will expand the

application of this modified polymer film in electrochemical field for the determination of other drugs.

Acknowledgment

Financial support from Taif University, is gratefully acknowledged.

References

1. S. C. Sweetman, "*Martindale", The complete Drug Reference*, 36th ed.,Pharmaceutical Press, London, (2009)1209.
2. M. C. F. Ferraro, P. M. Castellano, and T. S. Kaufman, *J. Pharm. Biomed. Anal*., 34 (2004) 305.
3. M. C. F. Ferraro, P. M. Castellano, and T. S. Kaufman, *J. Pharm. Biomed. Anal*., 30 (2002) 1121.
4. P. Ortega-Barrales, G. Pellerano, F. A. Vazquez, and A. Molina-Diaz, *Anal. Letters*, 35 (2002) 1491.
5. M. C. F. Ferraro, P. M. Castellano, and T. S. Kaufman, *J. Pharm. Biomed. Anal*., 26 (2001) 443.
6. D. Ivanovic, M. Medenica, S. Kojic-Marinkovic, and D. Radulovic, *J. Pharm. Biomed*. Anal., 23 (2000) 965.
7. R. A. S. Lapa, J. L. F. C. Lima, and J. L. M. Santos, *Anal. Chim. Acta*, 407 (2000) 225.
8. M. Kartal, and N. Erk, *J. Pharm. Biomed. Anal*., 19 (1999) 477.
9. C. V. N. Prasad, C. Parihar, K, Sunil, and P. Parimoo, *J. Pharm. Biomed. Anal*., 17 (1998) 877.
10. M. B. Devani, S. S. Pandya, and S. A. Shah, *Indian J. Pharm. Sci*., 53(1991) 96.
11. C. S. P. Sastry, M. V. Suryanarayana, and A. S. R. P. Tipirneni, *Talanta*, 36 (1989) 491.
12. C. S. P. Sastry, T. N. V. Prasad, A. R. M. Rao, and E. V. Rao, *IndianDrugs*, 25 (1988) 206.
13. C. S. P. Sastry, T. N. V. Prasad, B. S. Sastry, and E. V. Rao, *Analyst*, 113 (1988) 255.
14. R. Solanki, *Inter. J. Advan. Pharm. Anal*., 1 (2011) 16.
15. B. P. Nagori and R. Solanki, *Indian J. Pharm. Sci*., 72 (2010) 384.
16. C. S. Myung, J. W. Bae, Y. S. Park, C. I. Choi, Y. Song, J. H. Sa, C. G.Jang, and S. Y. Lee, *J. Liq. Chromat. Rel. Techn*., 31 (2008) 2455.
17. D. Thieme, J. Grosse, R. Lang, R. K. Mueller, and A. Wahl, *J. Chromat. B. Biomed. Appl*., 757 (2001) 49.
18. M. Zecevic, L. Zivanovic, S. Agatonovic-Kustrin, and D. Minic, *J. Pharm. Biomed. Ana*l., 24 (2001) 1019.
19. A. Rosado-Maria, A. I. Gasco-Lopez, A. Santos-Montes, and R.Izquierdo-Hornillos, *J. Chromat. B. Biomed. Appl*., 748 (2000) 415.

20. M. Zecevic, L. J. Zivanovic, S. Agatonovic-Kustrin, D. Ivanovic, and M. Maksimovic, *J. Pharm. Biomed. Anal*., 22 (2000) 1
21. S. Agatonovic-Kustrin. M. Zecevic, and L. J. Zivanovic, *J. Pharm. Biomed. Anal*., 21 (1999) 95.
22. S. Carda-Broch, J. S. Esteve-Romero, and M. C. Garcia-Alvarez-Coque, *Anal. Chim. Acta*, 375 (1998) 143.
23. S. Agatonovic-Kustrin. M. Zecevic, and L. J. Zivanovic, and I. G. Tucker, *J. Pharm. Biomed. Anal*., 17 (1998) 69.
24. S. Agatonovic-Kustrin. M. Zecevic, and L. J. Zivanovic, and I. G. Tucker,*Anal. Chim. Acta*, 364 (1998) 265.
25. A. Dominguez-Vidal, P. Ortega-Barrales, and A. Molina-Diaz, *Talanta*, 56 (2002) 1005.
26. Y. Z. Cao, C. Y. Mo, J. G. long, H. Chen, H. L. Wu, and R. Q. Yu, *Anal. Sci*., 18 (2002) 333.
27. J. A. Murillo-Pulgarin, A. Alanon-Molina, and P. Fernandez-Lopez, *Anal. Chim. Acta*, 449 (2001) 179.
28. J. A. Murillo-Pulgarin, A. Alanon-Molina, and P. Fernandez-Lopez, *Anal. Biochem.*, 292 (2001) 59.
29. J. A. Murillo-Pulgarin, A. Alanon-Molina, and P. Fernandez-Lopez, *Luminescence*, 15 (2000) 100.
30. J. A. Murillo-Pulgarin, A. Alanon-Molina, and P. Fernandez-Lopez,*Analyst*, 122 (1997) 247.
31. M. H. Abdel-Hay, S. M. Galal, M. M. Bedair, A. A. Gazy, and A. A. M. Wahbi, *Talanta*, 39 (1992) 1369.
32. M. I. Maguregui, R. M. Jimenez, and R. M. Alonso, *J. Chromatogr. Sci*., 36 (1998) 516.
33. E. Gonzalez, A. Beerra, and J. J. Laserna, *J. Chromatogr. B. Biomed. Appl*., 687 (1996) 145.
34. S. A. Halvatzis, A. M. Mihalatos, L. P. Palilis, and A. C. Calokerinos, *Anal. Chim. Acta*., 290 (1994) 172.
35. A. R. Allafchian, and A. A. Ensafi, *J. Braz. Chem. Soc*., 21 (2010) 564.
36. A. A. Ensafi, and A. R. Allafchian, *J. Pharm. Biomed. Anal*., 47 (2008)802
37. E. Hammam, *J. Pharm. Biomed. Anal*., 34 (2004) 1109.
38. G. B. El-Hefnawy, I. S. El-Hallag, E. M. Ghoneim, and M. M. Ghoneim, *J.Pharm. Biomed. Anal*., 34 (2004) 899
39. M. E. Martin, O. M. Hemandez, A. I. Jimenez, J. J. Arias, and F. Timenez,*Anal. Chim. Acta*, 38 (1999) 247.
40.H.T.S.Britton,Hydrogen Ions,fourth ed.,Chapman&Hall,London,1952,113 pp.
41. P. B. Desai, A. K. Srivastava, Sensors and Actuators B, 169 (2012) 341–348.

42. S. I. M. Zayed, H. A. M. Arida, Int. J. Electrochem. Sci., 8 (2013) 1340.
43. A. Allafchian, A. A. Ensafi, J. Braz. Chem. Soc., 21 (2010), 564-570.
44. E. Mirmomtaz, Ali A. Ensafi, S. Soleimanian-Zad. Electrochimica Acta 54 (2009) 1141–1146