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Preparation of Carbon Paste Electrodes and Its Using in Voltammetric Determination of Amiloride Hydrochloride Using in the Treatment of High Blood Pressure

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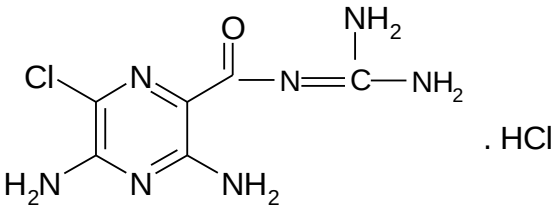
The anodic voltammetric behavior of amiloride hydrochloride (AmilCl) was studied at carbon paste electrode in 0.04 M Britton-Robinson buffer pH 2.96 using cyclic and differential pulse voltammetric techniques. Cyclic voltammetric study indicates that the oxidation process is irreversible and controlled by adsorption. Parameters affecting on the oxidation of AmilCl are optimized. Under the optimal conditions the oxidation peak current is proportional to concentration of AmilCl in the range 0.60-4.23 µg/ml AmilCl with detection and quantitation limits of 0.26 and 0.87 µg/ml AmilCl, respectively. The proposed method was successfully applied for the assay of AmilCl in Moduretic tablets.

Keywords: Amiloride hydrochloride; Carbon paste electrodes; Anodic adsorptive stripping voltammetry; Pharmaceutical dosage form.

1. INTRODUCTION

Amiloride hydrochloride, (AmilCl), N-amidino-3,5-diamino-6-chloropyrazine-2-carboxamide hydrochloride [2016-88-8] (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].



Scheme I. Structural formula of amiloride hydrochloride

Several analytical methods have been described in the literature for the assay of amiloride hydrochloride, including, spetrophotometry [2-13], high performance liquid chromatography [8, 14-24], fluorimetry [25-31] , capillary zone electrophoresis [32,33], chemiluminometric [34], potentiometry [35,36]. Although the electrochemical reduction of this drug have been studied [37-39], the electrochemical oxidation of this drug has not been previously reported. This work aimed to study the oxidation of this drug at carbon paste electrode 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 were of analytical grade. Double distilled water was used throughout all experiments. 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., graphite powder (1-2 micron) from Aldrich. and paraffin oil from Merck. As a supporting electrolyte, a series of 0.04 M Britton-Robinson (BR) buffer pH 2.0-11.5 (a mixture of each of acetic, orthophosphoric and boric acids), adjusted to the required pH with 0.2 M sodium hydroxide was prepared.

2.2. Apparatus

All voltammetric measurements were performed using Metrohm 797 VA Computrace (Herisau, Switzerland) equipped with a Metrohm VA 694 stand. Three electrodes assembly cell consisted of carbon paste electrode (CPE) as working electrode, an Ag/AgCl in 3 mol/L KCl (Metrohm 6.0728.000) as a reference electrode and platinium wire (Metrohm 6.0343.000) as an auxiliary electrode. The pH measurement were carried out with Hanna pH 211 microprocessor pH meter.

2. 3. Preparation of carbon paste electrode

The carbon paste was prepared by thoroughly mixing 5 g of graphite powder with 1.8 ml of paraffin oil in a mortar with pestle. The carbon paste was packed into the hole of the electrode body

and smoothed on a clean paper until it had a shiny appearance. The electrode body was constructed by pressing a small rode of stainless steel (diameter 2 mm) inside a micropipette tip (1 ml volume capacity) leaving a depression at the surface tip approximately 1 mm for housing the carbon paste, and a thin wire was inserted through the opposite end to establish electrical contact.[40] The carbon paste electrode was immersed in the supporting electrolyte placed in the cell and several sweeps were applied to obtain a low background current.

2.4. Procedure

A 10 ml 0.04 M BR buffer pH 2.96 solution was introduced into the voltammetric cell, then a known amount of the drug solution was pipetted into the cell. The differential pulse technique was applied by scaning from 0 to 1.4 V with scan rate of 50 mVs^{-1}, and pulse amplitude of 50 mV.

2.5. Determination of AmilCl in Moduretic tablets (5 mg amiloride hydrochloride, 50 mg hydrochlorothiazide/tablet)

Twenty tablets were accurately weighed, and powdered in a mortar. The required amount from the crushed tablets powder was dissolved in about 30 ml of bidistilled water and filtered in a 100 ml measuring flask. The residue was washed three times with bidistilled water, and the volume was completed to the mark by the same solvent. A 10-ml volume of 0.04 M Britton-Robinson buffer pH 2.96 was introduced into the voltammetric cell and suitable volume of the above tablets solution is pipetted into the buffer in the voltammetric cell; the procedure is repeated as described. The nominal content of the tablets is calculated using standard addition technique.

3. RESULTS AND DISCUSSION

3. 1. Cyclic voltammetric studies

Fig. 1 illustrates the repeatative cyclic voltammograms for $6.54x10^{-5}$ M AmilCl, $2H_2O$ solution in 0.04 M BR buffer pH 2.96, scan rate = 50 mVs^{-1}**,** and accumulation potential E_a= 0V. Oxidation peak appears at 1.19 V, which may be due to oxidation of the amino group of the drug, and no reduction peak is observed in the cathodic branch which suggests that the process is irreversible.

The repeatative cyclic voltammograms show that the peak current decreases in the second and third cycles, and this behavior gives an indication of an adsorption character. A plot of logarithm of peak current versus logarithm of the scan rate gave a straight line relation with a slope of 0.94 which is close to the theoretically 1.0 for an ideal relation of surface species [41]. The peak potential shifited to more positive values with increasing scan rate.

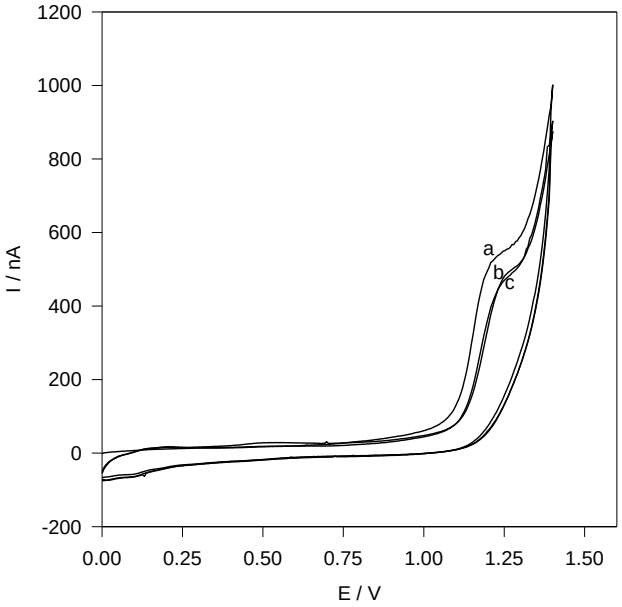


Figure 1. Successive cyclic voltammograms of 6.54×10^{-5} M AmilCl solution in 0.04 M BR buffer pH 2.96, and scan rate of 50 mVs^{-1}

3.2. DP voltammetric studies

DP voltammetry is effective and rapid electroanalytical technique with well established advantages, including good discrimination against background current and low detection limits [42]. Various supporting electrolytes such as acetate buffer, phthalate buffer, BR buffer, and sodium perchlorate were tested. The best results with respect to sensitivity accompanied with sharper response were obtained in the case of BR buffer, so this buffer was selected for further work. The effect of pH on the peak current and oxidation potential were tested over the pH range 1.86 – 11.0 (Fig. 2).

The peak current reach its maximum at pH 2.96, so this pH value was therefore adopted in the following experiments.

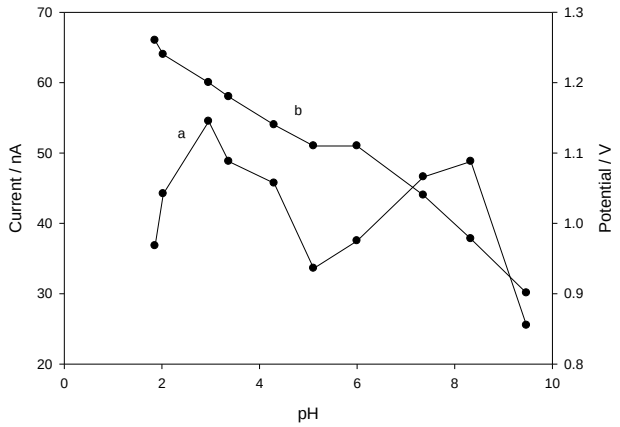


Figure 2. Effect of pH on the DP anodic peak current (a) and peak potential (b) of 1.9×10^{-6} M AmilCl in 0.04 M BR buffer, accumulation potential = 0 V, accumulation time = 30 s, scan rate = 50 mV/s and pulse amplitude = 50 mV.

The anodic peak potential shifts towards less positive values with increasing the pH, indicating that the protons are involved in the electrode reaction process. The plot of peak potential versus pH exhibits two linear intervals in the pH ranges of 1.86-5.11 and 6.00-9.47 with slopes of -45.0 and -60.0 mV per pH unit. The breaks at pH 5.11 and 6.00 may be correlated to the pk_a of the drug. The effect of concentration of BR buffer (0.02, 0.04 and 0.1 M) on the peak current indicated that the highest peak current was obtained at 0.04 M BR buffer.

The effet of accumulation potential on the oxidation peak current was studied for $1.9x10^{-6}$ M AmilCl at 30 s accumulation time, 50 mV/s scan rate, and 50 mV pulse amplitude, the current peak was nearly constant on changing the accumulation potential (E_a) from 0 to 900 mV.

The effect of accumulation time on the oxidation peak current was studied at two concentrations levels $2x10^{-6}$ and $4.98x10^{-6}$ M AmilCl (Fig. 3). The current increases linearly with increasing the accumulation time (t_a), indicating that the longer the accumulation time, the increase the drug concentration at the electrode surface, and the larger the peak current, then as the accumulation time increases the peak current tends to level off. 60 s accumulation time was generally used for subsequent studies.

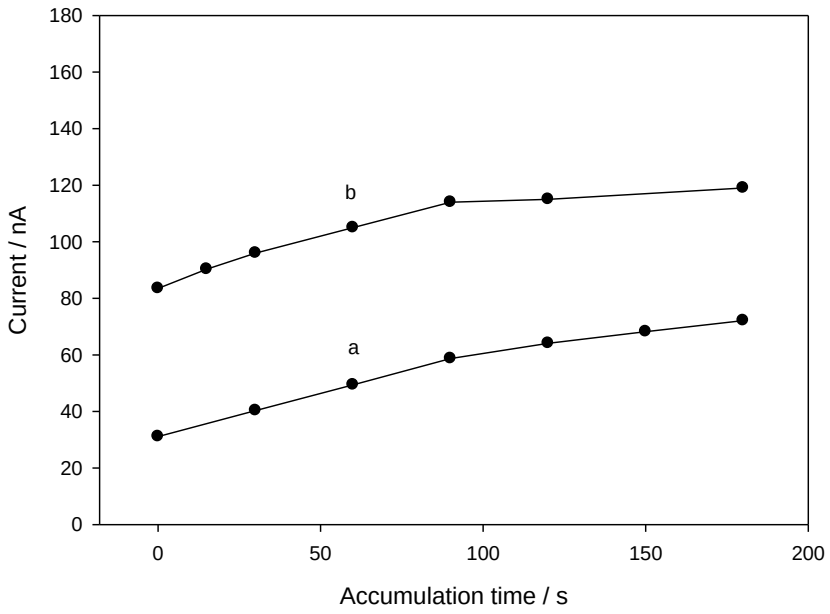


Figure 3. Effect of accumulation time on the peak current for a, $2x10^{-6}$ Mand b, $4.98x10^{-6}$ M AmilCl in 0.04M BR buffer pH 2.96 at accumulation potential = 0 V, scan rate = 50 mV/s, and pulse amplitude = 50 mV

Instrumental parameters such as pulse amplitude and scan rate were also optimized. Variations of pulse amplitude (10-100 mV) and scan rate (10-100 mVs^{-1}) at $1.9x10^{-6}$ M AmilCl, and 60 s accumulation time were examined. The results shows that a pulse amplitude of 50 mV and a scan rate of 50 mVs^{-1} produced the best peak in intensity and shape.

3.3. 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 0.60-4.23 µg/ml AmilCl. Fig. 4 represents the differential pulse anodic voltammograms recorded using the standard addition method. The linear regression equation was I (nA) = 41.15+29.82C (µg/ml), with a correlation coefficient of o.9982, the limit of detection (LOD = 3(SDa)/b) and limit of quantitation (LOQ = 10(SDa)/b), were calculated [43], where SDa is the standard deviation of the intercept and b is the slope of the calibration graph. LOD and LOQ were found to be 0.26 and 0.87 µg/ml AmilCl, respectively. 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

Parameter	
Linear range, µg/ml	0.60-4.23
Slope	29.82
Intercept	41.15
Correlation coefficient (r)	0.9982
LOD, µg/ml	0.26
LOQ, µg/ml	0.87

3.4. Reproducibility

The intra-day and inter-day (day-to-day) precision expressed as relative standard deviations were 0.43 and 1.09%, respectively.

3.5. Interference

The effect of interference from excipients usually present in pharmaceutical formulations was examined. No interference (< 2.9% change in oxidation current), was observed in the presence of 100 fold excess of lactose, talc, starch or magnesium stearate. These results clearly demonstrated that the proposed method had a reasonable selectivity. These ingredients are electroinactive and its adsorption onto the carbon paste electrode is very limited under the optimum procedure conditions [44]

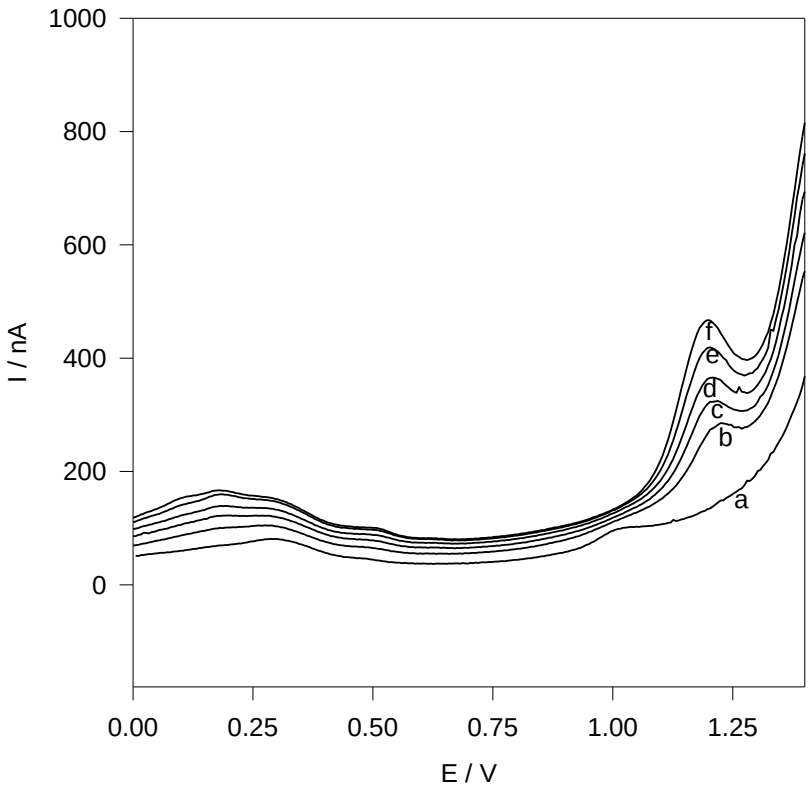


Figure 4. Differential pulse voltammograms for different concentrations of amilCl in 0.04 M BR buffer pH 2.96, accumulation time 60 s, scan rate of 50 mVs^{-1}, pulse amplitude 50 mV

3.6. Determination of AmilCl in Moduretic tablets

Table 2. Statistical comparison between the results of Moduretic tablets using the proposed method and the reference method.

Parameter	Proposed method	Reference method[8]
Mean recovery, %	98.20	97.79
SD	1.294	1.009
RSD, %	1.318	1.032
F-ratio (9.28)[a]	1.645	
t-test (2.447)[b]	0.500	

Average of four determinations for the proposed and reference methods.

[a] Tabulated F-value at 95% confidence level.

[b] Tabulated t-value at 95% confidence level and 6 degrees of freedom.

The proposed DP voltammetric method was successfully applied for the assay of AmilCl in Moduretic tablets (5 mg amiloride hydrochloride, 50 mg hydrochlorothiazide/tablet). The mean

recovery and the relative standard deviation values are summarized in Table 2. The data indicate that there is no interference from the other drug, hydrochlorothiazide, and from the excipients used in the formulations of the tablets. The results of the proposed method were compared with the results of the HPLC referenc method [8], by means of Student's t- and F-ratio tests at 95% confidence level [45], there is no significant difference in accuracy or precision between the two methods.

4. CONCLUSIONS

In this paper, the electrochemical behavior of amiloride hydrochloride on carbon paste electrodes has been investigated by cyclic and differential pulse voltammetric techniques. The proposed procedure showed clear advantges, such as no pre-treatment or time consuming extractions steps were required prior to the analysis, ease of preparation and easy renewable of the electrode surface. The proposed method is less expensive than alternative techniques like HPLC, and hence can be applied to the routine determination of the drug in quality control laboratories.

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