Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
34 (2004) 1109–1116
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Behavior and quantification studies of amiloride drug using cyclic and square-wave adsorptive stripping voltammetry at a mercury electrode

E. Hammam*

Faculty of Science, Department of Chemistry, Tanta University, 31527 Tanta, Egypt

Received 22 August 2003; received in revised form 18 November 2003; accepted 20 November 2003

Abstract

The cyclic voltammograms of amiloride at the hanging mercury drop electrode showed a single well-defined four-electron irreversible cathodic peak in Britton–Robinson (B–R) buffer of pH 2. At higher pH values (pH ≥3), two irreversible cathodic peaks corresponding to the transfer of four (first peak) and two (second peak) electrons, were obtained The peak potentials were shifted to more negative values on the increase of pH of the medium, implying the involvement of protons in the electrode reaction and that the proton-transfer reaction precedes the proper electrode process. The 4-electron single peak (pH 2) or the first peak (pH ≥ 3) may be attributed to the cleavage of the –CH=NH double bond of the *N*-imidino amide group with the release of NH_3 molecule. While the second peak may be due to the saturation of the C=O double bond of the carboxamide moiety.

Based on the interfacial adsorptive character of the drug onto the mercury electrode surface, a simple, sensitive and low cost square-wave adsorptive cathodic stripping (SWAdCS) voltammetric procedure was optimized for analysis of the drug. The optimal operational conditions of the proposed procedure were: accumulation potential $E_{acc} = -0.7$ V, accumulation time $t_{acc} = 60$–65 s, scan increment = 10 mV, pulse-amplitude = 50–60 mV, frequency = 120 Hz using a B–R buffer of pH 8 as a supporting electrolyte. The linear concentration range was found to be 2×10^{-9} to 2×10^{-7} M amiloride with limits of detection (LOD) and quantitation (LOQ) of 1.9×10^{-10} and 6.3×10^{-10} M, respectively. The procedure was successfully applied for determination of amiloride in pharmaceutical formulation and spiked in human serum. The LOD and LOQ of amiloride spiked in human serum were 5.7×10^{-10} and 1.9×10^{-9} M amiloride, respectively. The procedure did not require sample pretreatment or any time-consuming extraction or evaporation steps, other than deproteinization and then centrifugal separation of protein from serum sample prior to analysis of the drug.

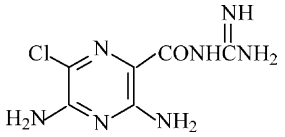
Keywords: Amiloride; Yostiretic® tablets; Spiked human serum; Quantification; Square-wave adsorptive stripping voltammetry

1. Introduction

Amiloride, *N*-amino-3,5-diamino-6-chloropyrazine-2-carboxamide (Scheme 1): is a potassium-conserving

* Tel.: +20-40-3343398; fax: +20-40-3350804.
E-mail address: essamhammam2k@hotmail.com (E. Hammam).

0731-7085/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2003.11.011



Scheme 1.

relatively weak natriuretic with anti-hypertensive activity. It is a therapeutic drug and a pharmacological tool usually used in combination with thiazid diuretics or other kaliuretic-diuretic agents in congestive heart failure or hypertension [1].

Amiloride is also used for the management of edema including edema associated with congestive heart failure, hepatic cirrhosis, and hyperaldosteronism. It could also be misused in sports involving weight categories to obtain a rapid diminution of weight or to increase the production of urine, which would reduce the concentration and thus the possibility of detection of a urine specimen in drug test [2].

Amiloride was individually determined in biological fluids, utilizing isopotential fluorimetry [3], fast flow-injection fluorimetry [4], capillary-zone electrophoresis [5], high-performance liquid chromatography [6–8], solid-phase spectrophotometry [9] and dual-stopped-flow spectrophotometry [10].

Different methods were reported for the determination of amiloride in presence of hydrochlorothiazide or other drugs in pharmaceutical formulation and biological fluids. These include spectrophotometry [11–16], fluorimetry [17,18], capillary isotachophoresis [19], continuous-flow chemiluminescence [20], colorimetry [21], high-performance liquid chromatography [22,23] and differential-pulse polarography [24]. Most of the reported analytical methods for quantitation of amiloride, individually or in presence of other drugs, did not achieve sufficient enough quantitation limits and/or most of them required sample pretreatment or time-consuming extraction or evaporation steps prior to analysis of the drug.

Adsorptive stripping analysis greatly enhances the scope of stripping measurements towards various organic compounds. The high sensitivity and low cost of stripping analysis have led to its application in a large number of analytical problems [25]. This technique did not require sample pretreatment or time-consuming extraction or evaporation steps prior to analysis of the drug.

This paper aimed to study the voltammetric behavior of amiloride and its interfacial accumulation onto the hanging mercury drop electrode. Besides, a simple, sensitive and low cost square-wave adsorptive stripping (SWAdCS) voltammetric procedure was developed for the direct trace determination of amiloride in pharmaceutical formulation and spiked human serum samples.

2. Experimental

2.1. Instrumentation

Voltammetric measurements were carried out employing the electrochemical analyzers models 394 and 263A-PAR. The electrode assembly 303A-PAR and a dark micro-electrolysis cell comprising of a hanging mercury drop electrode (HMDE) as a working electrode (surface area $= 0.026\,cm^2$), an $Ag/AgCl/KCl_s$ as a reference electrode and a platinum wire as an auxiliary electrode, were used. A magnetic stirrer (305-PAR) and a stirring bar provided the convective transport during the preconcentration step. The peak heights were automatically measured using the "tangent fit" capability of the instrument.

An Eppendorf centrifuge 5417C was used for separation of the precipitated proteins from human serum samples prior to assay of the drug. A digital micro-pippetter (Volac) was used for transfer of the solutions during the present electrochemical measurements.

2.2. Reagents and solutions

All reagents were of the analytical-reagent grade. A mettler balance (Toledo AB104) was used for weighing the solid materials. De-ionized water was obtained from a Purite Still Plus HP de-ionizer attached to a Hamilton AquaMatic bi-distillation water system.

A standard stock solution (1×10^{-3} M) of amiloride (Sigma) was prepared by dissolving an accurate mass of the bulk drug in an appropriate volume of ethanol, then stored in dark at 4 °C. More dilute solutions (10^{-6} to 10^{-4} M) were prepared daily by accurate dilution with ethanol just before use. Amiloride solutions were stable and their concentrations did not change with time.

B–R buffers of pH 2–11 used as supporting electrolyte were prepared in de-ionized water in the usual way [26].

2.3. Samples solutions

Ten Yostiretic® tablets (labeled as to containing 5 mg amiloride and 50 mg hydrochlorothiazide/tablet, Amoun Co., Egypt) were weighed and the average mass per tablet was determined, then ground to a homogeneous fine powder in a mortar. A portion of the finely ground material was accurately weighed and transferred into a 100 ml-calibrated flask contained 50 ml ethanol. The content of the flask was sonicated for about 15 min and then made up to the volume with ethanol. The solution was next filtered through a 0.45 μm milli-pore filter. The desired concentration was obtained by accurate dilution with ethanol.

Human serum samples were obtained from healthy volunteers and stored frozen until the assay. An aliquot of the standard stock amiloride solution was fortified with the human serum sample. A 100 μl aliquot of this solution was diluted to a 1.0 ml volume with ethanol in a 2 ml volume centrifuge tube. The precipitated proteins were separated by centrifugation for 3 min at 14,000 rpm. The clear supernatant layer was a protein-free spiked human serum solution.

2.4. General analytical procedure

Ten milliliters of B–R buffer and an appropriate volume of the drug solution were introduced into the micro-electrolysis cell, through which pure nitrogen stream was passed for 5 min before recording the voltammogram. A selected accumulation potential was applied at the HMDE for a selected time, while the solution was stirred. At the end of the accumulation time period the stirring was stopped and 10 s were allowed for the solution to become quiescent. Then, the voltammogram was recorded by scanning the potential toward the negative direction using the square-wave mode. Calibration graphs were constricted. The mean percentage recoveries and standard deviations were estimated using both the calibration curves and standard addition methods. All data were obtained at room temperature.

3. Results and discussion

3.1. Cyclic voltammetric studies

In general, the pH of the electrolysis medium is one of the variables that commonly and strongly influences the shape of the voltammogram, and therefore it was important to investigate the effect of pH on the electrochemical behavior of the drug. The cyclic voltammograms for 1×10^{-4} M amiloride at the HMDE showed a single well-defined irreversible cathodic peak in B–R buffer of pH 2. At higher pH values (pH $\geq$ 3) two irreversible cathodic peaks were obtained. Controlled-potential coulometric microanalysis of amiloride solutions of different concentrations in B–R buffers, revealed that four electrons were consumed for reduction of the drug via a single cathodic peak at pH 2. While in B–R buffers of pH 3–11, four and two electrons were consumed for reduction of the drug via two steps, the first and second peaks, respectively. Accordingly, the single peak (pH 2) or the first peak (pH $\geq$ 3) may be attributed to the cleavage of the –CH=NH double bond of the *N*-imidino amide group with the release of NH_3 molecule. While the second peak (pH $\geq$ 3) may be due to the saturation of the C=O double bond of the carboxamide moiety.

The increase of pH generated shift of the peak potentials to more negative values, implying the involvement of protons in the electrode reaction and that the proton-transfer reaction precedes the electrode process proper [27]. The peak potentials were also displaced to more negative values with the increase of the scan rate (ν), indicating the irreversible nature of the electrode reaction. The plots of $\log i_p$ versus the $\log \nu$ for both peaks in B–R buffers of pH 3–11 were straight lines. Their slope values ranging from 0.52 to 0.57, which are close to the theoretical value of 0.5 that expected for ideal reaction of solution species [28], so the reduction process of amiloride at the HMDE had mainly a diffusive component. On the other side, the co-formulated hydrochlorothiazide was found to be electro-inactive under the present experimental conditions, since no peak appeared for it whatever present, single or mixed with the amiloride in the electrolysis cell and then, it does not interfere in the voltammetric determination of amiloride.

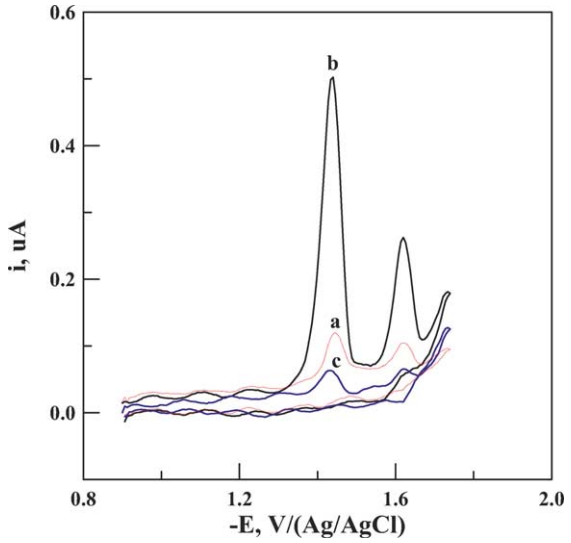


Fig. 1. Cyclic voltammograms for 2×10^{-7} M amiloride in B–R buffer of pH 8 at scan rate = 100 mV s^{-1}: (a) without preconcentration; (b) after preconcentration for 60 s at $E_{acc} = -0.9$ V; (c) its repetitive cycle at the same mercury drop.

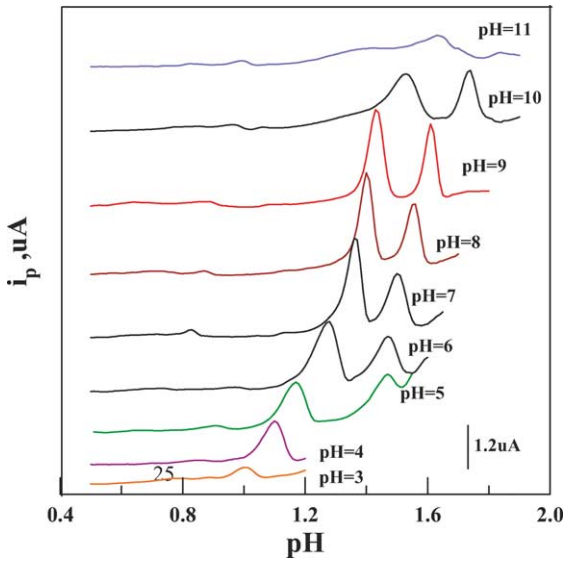


Fig. 2. SWAdCS voltammograms for 1.7×10^{-7} M amiloride in B–R buffers of different pH values, t_{acc} = 60 s, $E_{acc} = -0.5$ V, pulse-amplitude = 50 mV, scan increment = 10 mV and frequency f = 120 Hz. Other operational conditions are as those indicated in Table 1.

The interfacial adsorptive character of amiloride onto the HMDE was identified from the peak current (i_p) dependence, after preconcentration of the drug for 30 s in B–R. buffer of pH 5, upon the scan rate ν. The plots of log i_p versus log ν, gave a straight line following the equation: log i_p (μA) = 1.11 log ν (mV s^{-1}) + 0.3. The slope value of 1.11 is close to the theoretical value of 1.0 that is expected for an ideal reaction of surface species [28].

The interfacial adsorptive character of the drug was also identified from the voltammograms for 2×10^{-7} M amiloride in B–R. buffer of pH 8, which were recorded without preconcentration (Fig. 1, curve a) or following preconcentration of the drug for 30 s (Fig. 1, curve b). After preconcentration of the drug for short time period two well-defined peaks were obtained, the first peak was much more developed and sharp. This behavior may be attributed to the accumulation of the drug onto the HMDE surface during the preconcentration step. A substantial decrease of the cathodic peak current was observed in subsequent scan at the same mercury drop (Fig. 1, curve c), indicating a rapid desorption of the drug out of the mercury electrode surface.

3.2. Square-wave stripping voltammetric studies

3.2.1. Effect of pH

The influence of pH on the square-wave voltammetric response for 1.7×10^{-7} M amiloride was examined in B–R buffers of pH 3–11, after preconcentration of the drug onto the HMDE for 60 s. A single irreversible peak was generated in solutions of pH $\leq$ 4, while two cathodic irreversible peaks were obtained over the pH range 5–10. The first peak recorded within the pH range 7–8 was much more developed and sharp, compared to both the single and the second peaks (Fig. 2). Therefore, the first peak obtained in B–R buffer of pH 8 was considered in the present analytical study.

3.2.2. Effect of accumulation potential and time

The effect of accumulation potential on the SWAdCS voltammetric current of the 1st peak of amiloride was investigated after preconcentration of the drug onto the HMDE for 60 s over the potential range −0.1 to −1.0 V (versus Ag/AgCl/KCl_s). A much more developed peak current was achieved over the potential range −0.6 to −0.7 V (Fig. 3). So,

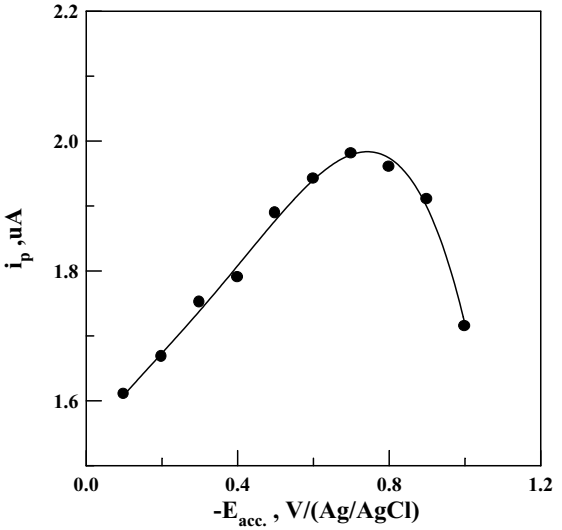


Fig. 3. Effect of accumulation potential on the SWAdCS voltammetric peak current for 1.7×10^{-7} M amiloride in B–R buffer of pH 8. Operational conditions are as those indicated in Fig. 2.

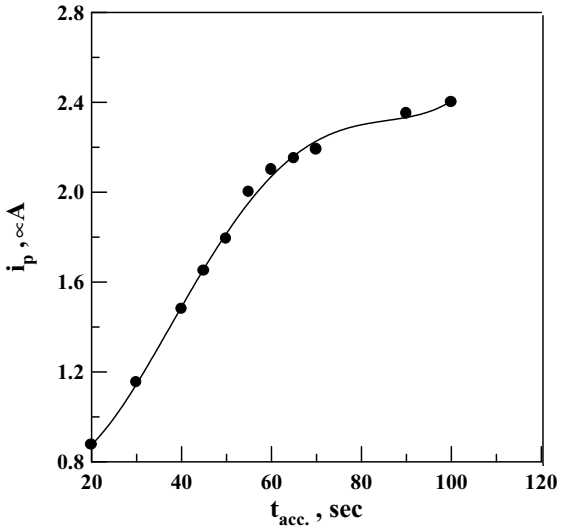


Fig. 4. Effect of accumulation time on the SWAdCS voltammetric peak current for 1.7×10^{-7} M amiloride in B–R buffer of pH 8. Operational conditions are as those indicated in Fig. 2.

a preconcentration potential of −0.7 V was chosen over the rest of the study.

The effect of accumulation time period on the SWAdCS voltammetric current of the 1st peak for 1.7×10^{-7} M amiloride in B–R buffer of pH 8 was also investigated. As shown in Fig. 4, accumulation time period of 60–65 s at −0.7 V generated a much more peak current.

3.2.3. Effect of instrumental parameters

The influence of several instrumental parameters known to affect the square-wave stripping current response at the HMDE, such as frequency, scan increment, pulse-amplitude, mercury drop size, stirring rate, rest period and purge time were optimized. For this study, each variable was changed while the others were kept constant The variables of interest were studied over the ranges 10–120 Hz for frequency, 2–10 mV for scan increment and 30–80 mV for pulse-amplitude. The optimal values of these parameters were then chosen from the study of the variation of the peak current (i_p) of 1.7×10^{-7} M amiloride in B–R buffer of pH 8 with frequency *f*, scan increment ΔE and pulse-amplitude *a*. The peak current of amiloride was found to increase linearly on increase both the frequency *f*, and scan increment. On the other hand, the peak current increased on increasing the pulse-amplitude up to 50 mV. According to the foregoing studies, the optimal operational parameters of the developed procedure are reported in Table 1.

3.2.4. Validation of the procedure

The peak current (i_p) measured by means of the proposed SWAdCS voltammetric procedure was linearly related to the amiloride concentration over the range

Table 1
The optimal operational parameters of the proposed procedure for determination of amiloride in different samples

Parameter	Optimal value
pH	8
Electrolyte	Britton–Robinson buffer
Temperature (°C)	25
Preconcentration potential (V)	−0.7 V
Preconcentration time (s)	60–65
Linear concentration range (M)	2×10^{-9} to 2×10^{-7}
Mercury drop size (cm^2)	0.026
Stirring rate (rpm)	400
Purge time (min)	5
Rest time (s)	10
Frequency (Hz)	120
Scan increment (mV)	10
Pulse-amplitude (mV)	50–60

2×10^{-9} to 2×10^{-7} M. The regression equation of the calibration curve was: i_p (μA) = 17.49C (nM) + 0.63, (correlation coefficient $r = 0.995$ and number of points $n = 12$). The mean percentage recoveries of amiloride based on the average of four replicate measurements using both the calibration graph and standard addition methods were found reproducible. The limits of detection (LOD) of amiloride was estimated, using the equation [29]: 3s/m, where s represents the standard deviation of the peak current and m represents the slope of the calibration curve, and found to be 1.9×10^{-10} M. Using the same equation [29], but based on the signal-to-noise ratio of 10 instead of three, the limits of quantitation (LOQ) was found to be 6.3×10^{-10} M. This level of precision is highly suitable for the routine quality control analysis of the drug in tablets and biological fluids.

As the pharmaceutical formulations contain excipients, the effect of the following substances: lactose, sacarose, glucose, fructose, gelatin and magnesium stearate was studied. The results indicated that the excipients did not interfere in the determination of amiloride, since good percentage recoveries for 2×10^{-8} M amiloride drug were achieved as: 101.5 ± 1.1 and 98.8 ± 1.3 in the absence and presence of the excipients, respectively. On the other hand, the co-formulated hydrochlorothiazide did not interfere, since it is electro-inactive under the experimental conditions. Accordingly, the proposed SWAdCS voltammetric procedure can be considered selective.

The robustness [30] of the proposed procedure was examined by studying the effect of variation of some of the neck operational conditions of the proposed procedure such as pH (7–8), preconcentration potential (−0.6 to −0.7 V), preconcentration time (60–65 s) and pulse-amplitude (50–60 mV). As shown in Table 2, the mean percentage recoveries based on four replicate measurements were not significantly affected and consequently the optimized procedure was reliable for assay of amiloride and could be considered robust.

The ruggedness [30] of the measurements was examined by applying the proposed procedure to assay of the drug using potentiostats of two different models: 394 (laboratory 1) and 263A (laboratory 2). The results shown in Table 2 due to laboratory to laboratory were found reproducible.

3.3. Applications

3.3.1. Assay of amiloride in tablets

The proposed procedure was successfully applied for assay of amiloride in its pharmaceutical

Table 2
Influence of variation of some of the operational parameters of the proposed procedure on the mean percentage recovery of 5×10^{-8} M amiloride; frequency = 120 Hz and scan increment = 10 mV

Parameters	R ± S.D.[a] (%)	Operational conditions
pH of the medium[b]		
7	99.1 ± 0.8	$E_{acc} = -0.7$ V, $t_{acc} = 60$ s
8	101.5 ± 0.9	$a = 60$ mV
Preconcentration potential (E_{acc})[b]		
−0.6 V	99.1 ± 0.6	pH = 8, $t_{acc} = 60$ s
−0.7 V	101.5 ± 0.9	$a = 60$ mV
Preconcentration time (t_{acc})[b]		
60 s	101.5 ± 0.9	pH = 8, $E_{acc} = -0.7$ V
65 s	99.3 ± 0.7	$a = 60$ mV
Pulse-amplitude (a)[b]		
50 mV	99.4 ± 0.3	pH = 8, $E_{acc} = -0.7$ V, $t_{acc} = 60$ s
60 mV	101.5 ± 0.9	
Potentiostat (PAR)		
Laboratory(1)—model 394	101.5 ± 0.9	pH = 8, $E_{acc} = -0.7$ V, $t_{acc} = 60$ s, $a = 60$ mV
Laboratory (2)—model 263 A	101.7 ± 1.1	

[a] Average of four replicate measurements.
[b] Using potentiostat model 394.

Table 3
Representative results of SWAdCS voltammetric assay of amiloride in Yostiretic® tablets using: (A) the calibration graph and (B) the standard addition methods; B–R buffer of pH 8, $t_{acc} = 60$ s, $E_{acc} = -0.7$ V, scan increment = 10 mV, pulse-amplitude = 60 mV and frequency = 120 Hz

Drug concentration in tablet (taken) ($\times 10^7$ M)	Average drug concentration (found)[a] ($\times 10^7$ M)	Mean % R[a]	RSD[a] (%)
(A)			
5	4.92	98.4	1.1
3	2.94	98.0	1.3
2	1.95	97.5	1.1
(B)[b]			
5	4.91	98.2	1.1
3	3.06	102.0	0.8
2	2.05	102.5	0.9

[a] Average of four replicate measurements.
[b] Measured with respect to a standard addition of 5×10^{-7} M amiloride.

formulation (Yostiretic® tablets). The mean percentage recoveries of the drug based on the average of four replicate measurements using both the calibration graph and standard addition methods were found reproducible (Table 3). The results were favorably compared to those obtained by a reported method [14].

3.3.2. Assay of amiloride spiked in human serum

The present optimized SWAdCS voltammetric procedure was also successfully applied for the determination of amiloride spiked in human serum (Fig. 5). The linear variation of (i_p) versus the amiloride concentration C (5×10^{-9} to 1×10^{-7} M) was represented by a straight-line equation: i_p (μA) = 16.6C (nM) + 0.55 ($r = 0.996$ and $n = 9$). The reliability of the procedure for the determination of different concentrations of amiloride spiked in human serum was checked by estimation of the mean percentage recoveries based on the average of four replicate measurements of the drug using both the calibration graph of the spiked human serum and the standard addition methods (Table 4). The mean percentage recoveries of the different amiloride concentrations spiked in human serum samples were found reproducible. The results were favorably compared to those obtained (98.2 ± 0.9 to 99.02 ± 1.3) by a reported method [14].

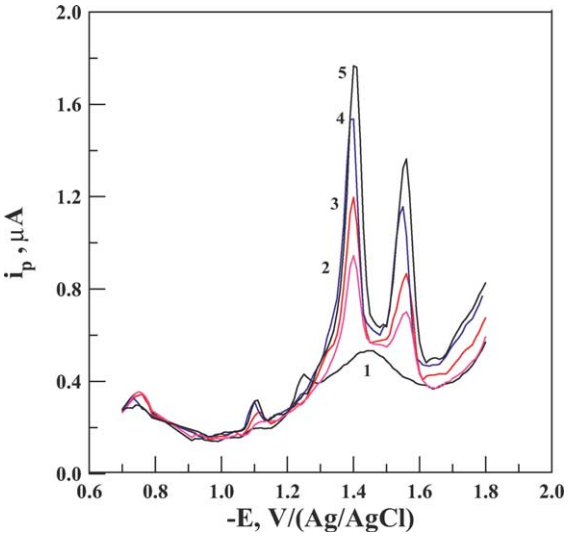


Fig. 5. SWAdCS voltammograms for different concentrations of amiloride spiked in human serum: (1) background; (2) 7×10^{-9}; (3) 2.1×10^{-8}; (4) 2.8×10^{-8}; and (5) 3.5×10^{-8} M in B–R buffer of pH 8; $t_{acc} = 60$ s, $E_{acc} = -0.7$ V, scan increment = 10 mV, pulse-amplitude = 60 mV and frequency = 120 Hz.

The LOD and LOQ of amiloride spiked in human serum were estimated and found to be 5.7×10^{-10} and 1.9×10^{-9} M, respectively. According to these results, the proposed procedure may be applied for determination of amiloride in real serum samples.

Table 4
Representative results of SWAdCS voltammetric assay of amiloride spiked in human serum using (A) the calibration graph and (B) the standard addition methods; B–R. buffer of pH 8, $t_{acc.} = 60$ s, $E_{acc.} = -0.7$ scan increment = 10 mV, pulse-amplitude = 60 mV and frequency = 120 Hz

Drug spiked in serum (taken) ($\times 10^8$ M)	Average drug concentration (found)[a] ($\times 10^8$ M)	Mean %R[a]	RSD[a] (%)
(A)			
1.2	1.23	102.5	1.6
3.3	3.35	101.5	1.2
4.5	4.55	101.1	0.9
(B)[b]			
2.0	1.95	97.5	0.7
3.0	3.04	101.3	1.1
4.0	4.06	101.5	0.8

[a] Average of four replicate measurements.
[b] Measured with respect to a standard addition of 2×10^{-8} M amiloride.

4. Conclusion

The voltammetric behavior of amiloride at the HMDE was studied in B–R buffers of pH 2–11. A simple, fast, sensitive, selective and low cost SWAdCS voltammetric procedure was developed for the trace determination of the drug in pharmaceutical formulation and spiked in human serum samples without the necessity for sample pretreatment, or any time-consuming extraction or evaporation steps, prior to analysis of the drug. The proposed procedure could be used in trace analysis and clinical laboratories.

References

[1] G.K. Mcevory (Ed.), AHFS Drug Information, American Society of Hospital Pharmacists, 1990, pp. 1481–1483.
[2] W.O. Foye, T.L. Lemke, D.A. Williams, Principles of Medical Chemistry, 4th ed., Wiliams & Wilkins, USA, 1995, pp. 405–406.
[3] J.A. Murillo-Pulgarin, A.A. Molina, P.F. Lopez, Analyst 122 (1997) 247–252.
[4] A. Dominguez-Vidal, P. Ortega-Barrales, A. Mollina-Diaz, Talanta 56 (2002) 1005–1013.
[5] S.E. Gonzalez, A. Becerra, J.J. Laserna, J. Chromatogr. B: Biomed. Appl. 687 (1996) 145–150.
[6] E.B. Domingo, M.J. Medinahernandez, G.R. Ramos, M.C.G. Alvarez-Coque, J. Chromatogr.-Biomed. 582 (1992) 189–194.
[7] H. Bi, S.F. Cooper, M.G. Cote, J. Chromatogr.-Biomed. 582 (1992) 93–101.
[8] E. Bechgaard, J. Chromatogr.-Biomed. 490 (1989) 219–223.
[9] P. Ortega-Barrales, G. Pelerano, A.A. Vazques, A. Molina-Dia, Anal. Lett. 35 (2002) 1491–1504.
[10] R.A.S. Lapa, J.L.F.C. Lima, J.L.M. Santos, Anal. Chim. Acta 407 (2000) 225–231.
[11] M.C.F. Ferraro, P.M. Castellano, T.S. Kaufman, J. Pharmacol. Biomed. Anal. 30 (2002) 1121–1131;
M.C.F. Ferraro, P.M. Castellano, T.S. Kaufman, J. Pharmacol. Biomed. Anal. 26 (2001) 443–451.
[12] N. Erk, F. Onur, Anal. Lett. 30 (1997) 1503–1515.
[13] M.H. Abdelhay, S.M. Galal, M.M. Bedair, A.A. Gazy, A.A.M. Wahbi, Talanta 39 (1992) 1369–1375.
[14] M. Kartal, N. Erk, J. Pharmacol. Biomed. Anal. 19 (1999) 477–485.
[15] E. Martin, O. Hernandez, F. Jimenez, J.J. Arias, Anal. Lett. 28 (1995) 1449–1464.
[16] M.I. Toral, S. Pope, S. Quintanilla, P. Richer, Int. J. Pharm. 249 (2002) 117–126.
[17] J.A.M. Pulgarin, A.A. Molina, P.F. Lopez, Anal. Biochem. 292 (2001) 59–68.
[18] J.A.M. Pulgarin, A.A. Molina, P.F. Lopez, Anal. Chim. Acta 449 (2001) 179–187.
[19] J. Sadecka, J. Polonsky, J. Chromatogr. A 735 (1996) 403–408.
[20] S.A. Halvatzis, A.M. Mihalatos, L.P. Palilis, A.C. Calokerinos, Anal. Chim. Acta 290 (1994) 172–178.
[21] M.M. Bedair, S.M. Galal, A.A. Gazy, A.A.M. Wahbi, Microchem. J. 50 (1994) 94–100.
[22] L.J. Zivanovic, M. Vasiljevic, A. Agatonovic-Kustrin, M. Maksimovic, Pharmacol. Biomed. Anal. 14 (1996) 1245–1250.
[23] M.J. Vandermeer, L.W. Brown, J. Chromatogr.-Biomed. 423 (1987) 351–357.
[24] M.E. Martin, O.M. Hernandez, A.I. Jimenez, J.J. Arias, F. Jimenez, Anal. Chim. Acta 381 (1999) 247–256.
[25] J. Wang, Stripping Analysis—Principles, Instrumentation, and Applications, Wiley–VCH, New York, 1985, pp. 9–59.
[26] H.T.S. Britton, Hydrogen Ions, 4th ed., Chapman & Hall, 1952 p. 113.
[27] P. Zuman, The Elucidation of Organic Electrode Processes, Academic Press, New York, 1969, pp. 21–22.
[28] E. Laviron, J. Electroanal. Chem. 112 (2002) 11–23.
[29] J.C. Miller, J.N. Miller Statistics for Analytical Chemistry, 4th ed., Ellis-Howood, New York, 1994, p. 115.
[30] The USA Pharmacopeia, The National Formularly, USP 26, 2003, p. 2442.