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ORIGINAL ARTICAL

Analysis of spironolactone residues in industrial wastewater and in drug formulations by cathodic stripping voltammetry

M.S. El-Shahawi*, A.S. Bashammakh, A.A. Al-Sibaai, E.A. Bahaidarah

Department of Chemistry, Faculty of Science, King Abdulaziz University, P.O.Box 80203, Jeddah 21589, Saudi Arabia

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Wastewater;
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Electrode mechanism

Abstract The redox behavior of spironolactone (SP) drug in Britton–Robinson (BR) buffer of pH 2–11 was investigated by differential pulse cathodic stripping voltammetry (DPCSV) and cyclic voltammetry (CV) at hanging mercury dropping electrode (HMDE). At pH 9–10.5, the DPCSV of SP drug showed two cathodic peaks at −1.15 and −1.38 V at the HMDE vs. Ag/AgCl reference electrode. In the CV, at pH 9–10, the dependence of the cathodic peak current, $I_{p,c}$ and peak potential, $E_{p,c}$ of the second peak ($E_{p,c2}$) on the scan rate (v) and on the depolizer (SP) concentrations was typical of an electrode coupled (EC) chemical reaction type mechanism. The plot of $I_{p,c}$ at −1.380 V of the DPCSV vs. SP concentration at pH 9 was linear over the concentration range of 1.2×10^{-10}–9.6×10^{-7} M. The lower limit of detection (LLOD) and limit of quantification (LOQ) of the drug were 1.1×10^{-11} and 4.14×10^{-11} M, respectively. The method was successfully applied for the analysis of SP residues in industrial wastewater, in pure form (98.2±3.1%) and in drug formulations e.g. Aldactone® tablet (98.35±2.9%).The method was validated by comparison with HPLC and the official data methods.

1. Introduction

Many drug residues have been found in water and the analysis of drug residues is the recent area and increasing its importance day by day [1,2]. Spironolactone (SP) chemically named as 7α-acetylthio-3-oxo-17α-pregn-4-ene-21,17β-carbolactone acid-γ-lactone is a steroid that acts as a competitive antagonist of the potent endogenous mineral-corticosteroid, aldosterone. It is a drug in a class of drugs called potassium-sparing diuretics (water pill). Such drug is widely used to treat high blood pressure, and fluid retention caused by various conditions, including heart disease. SP is indicated in the treatment of essential hypertension, edema associated with congestive heart failure, hepatic cirrhosis with

*Corresponding author at: Department of Chemistry, Faculty of Science, Damiatta University, Damiatta, Egypt. Tel.: +966 2 6952000; fax: +966 2 6952292.

E-mail addresses: malsaeed@kau.edu.sa, Mohammad_el_shahawi@yahoo.co.uk (M.S. El-Shahawi)

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ascites, the nephritic syndrome, idiopathic edema, and in diagnosis of primary aldosteronism [3–6].

Numerous methods e.g. chromatographic [7,8], spectrofluorimetric [9,10], spectrophoto-metric methods using univariate and multivariate calibration [11] and partial least-squares, multivariate calibration [12] have been reported for the analysis of SP in drug formulations, its metabolite canrenone, pure form human serum and urine. Most of these methods have insufficient sensitivity and selectivity for the trace levels of the drug in human serum and urine [11,12]. The low level of SP residues in wastewater samples was also not compatible with the detection limits of most of these methods [9–12]. Some of these methods are un-selective and require careful experimental conditions and time consuming [11,12].

Polarographic [13] and square-wave adsorptive cathodic stripping voltammetric (SW-AdSV) [14] have been developed for SP determination in drug formulations at dropping mercury electrode (DME) and hanging mercury drop electrode (HMDE) at pH 2–3, respectively. The selectivity and detection limit of polarographic method [13] are not compatible with the low level of SP residues in wastewater. On the other hand, in SW-ACSV method [14], peak resolution and selectivity of the observed cathodic peak at −1.15 V were also not compatible for analysis of SP residues in wastewater. In continuation to our previous work on analysis of drug residues in wastewater [15], this paper reports the redox behavior of SP drug in an attempt to develop a low cost and selective differential pulse cathodic stripping voltammetry (DPCSV) method for analysis of SP residues in wastewater and drug formulations. HMDE not only exhibited a strong adsorption towards SP but also provided remarkable stable and quantitatively reproducible analytical results. HMDE is safe as long as storage and its disposal is undertaken in a safe manner. HMDE is the only electrode type sensitive enough for metal speciation and drug residues in complex matrices e.g. natural water and wastewaters [16,17].

2. Experimental

2.1. Apparatus

A Metrohm 746 VA trace analyzer and 747 VA stand were used for recording the voltammetric measurements. A three-compartment (Metrohm) voltammetric electrochemical cell (10 mL) incorporating HMDE (0.38 mm^2) as a working electrode, double-junction Ag/AgCl, KCl (3.0 M) as a reference electrode and platinum wire (BAS model MW-1032) as a counter electrodes was used. Deionized water was supplied from Milli-Q Plus system (Millipore, Bedford, MA, USA). A digital pH-meter (model MP 220, Metter Toledo) and a digital-micro-pipette (Volac) were used for pH measurements and sample solutions.

2.2. Reagents

All chemicals used were of analytical reagent grade (BDH, Poole, England). Deionized water was used throughout. SP drug was obtained from Amriya Rhone-poulenc Pharmaceutical Industries Co. (Alexandria, Egypt). Stock solution of SP (2.4×10^{-3} M) was prepared in a minimum volume of ethanol and completed to the mark with deionized water. More diluted concentrations were prepared by diluting the stock solution with water. A series of BR buffers (pH 2.3−11.5) were used as supporting electrolytes. Aldactone® tablets (25 mg/tablet) were obtained from High Wycombe, England. Low density polyethylene (LDPE) bottles, Nalgene were used for storage of wastewater samples from municipal discharge station, Jeddah, KSA and stored at −20 °C in a refrigerator. The LDPE bottles were cleaned with hot detergent, HNO_3 (2.0 M) and HCl (0.5 M), and finally rinsed with water.

2.3. General DPCSV procedures

An accurate volume (10.0 mL) of an aqueous solution containing BR buffer at the required pH (2.1−11.5) was placed in the voltammetric cell. The solution was stirred and purged with N_2 gas for 10 min before recording the voltammograms. The stirrer was then stopped and after 10 s quiescence time, the DPCSV of the buffer was recorded by applying a negative going potential scan from 0.0 to −1.5 V vs. Ag/AgCl at a deposition potential of −0.45 V, accumulation time 60 s, starting potential 0.0 V, scan rate 60 mV/s and pulse amplitude −50 mV. After measurement of the blank solution, an accurate concentration (4.8×10^{-7} M) of SP was placed into the cell. The solution was stirred, purged with N_2 gas for 5 min and the voltammogram was recorded under the same experimental conditions of the supporting electrolyte. The influence of scan rates (v = 500–1000 mV/s), pH and SP concentration on the cyclic voltammetries (CVs) was also recorded at HMDE and Pt working electrodes.

2.4. Analytical applications

2.4.1. Analysis of SP in Aldactone® tablets

Ten tablets of Aldactone® (25 mg SP/tablet) were pulverized in a mortar, homogenized, accurately weighed and the average mass per tablet was then determined. An appropriate portion of the finally ground material was accurately weighed and dissolved in the minimum volume of ethanol in a sonicator for 20 min. The test solution was shaken for 15 min in a mechanical shaker to achieve complete dissolution of the active material and accurately transferred to a 25 mL measuring flask. The solution was completed to the mark with ethanol and an accurate volume (20.0 μL) of the clear supernatant liquor was transferred to the cell containing 10 mL of BR buffer at pH 9. The amount of the unknown SP drug in the test solution was then determined with the aid of standard curve constructed at $E_{p,c}$ = −1.4 V. Alternatively, the spiking method was also used as follows: An accurate volume (20 μL) of the supernatant liquor was transferred to the cell containing 10 mL of BR buffer at pH 9. Under the optimum conditions, the DPCSVs of the test solution before and after addition of various volumes of standard SP (10–50 μL in ethanol) were recorded. The current displayed at −1.40 V by the solution before and after addition of SP was measured and the unknown SP concentration was then computed from the linear plot of the standard addition.

2.4.2. Analysis of SP in water samples

Wastewater samples (200–300 mL) were collected from municipal discharge station samples, Jeddah city, KSA using a battery powered, peristaltic pump and immediately filtered through

0.45 μm cellulose membrane filters and stored in LDPE sample bottles (500 mL). An accurate volume (2.0 mL) of the test solution was transferred to the cell and the solution was completed to 10.0 mL with BR buffer (pH 9). The DPCSVs of the test solution before and after addition of standard SP (10–50 μL, 0.3 μg/mL in ethanol) were recorded. The current displayed at −1.38 V vs. Ag/AgCl electrode was measured and the concentration of the unknown sample was then determined from the linear plot of the spiked concentrations of SP vs. the corresponding cathodic peak current.

3. Results and discussion

3.1. Electrochemical behavior of SP drug

In BR buffer over a wide range of pH (2.1−11.5), the DPCSVs of SP (5×10^{-7} M) at the HMDE vs. Ag/AgCl electrode were investigated. Representative DPCSVs are shown in Fig.1. The DPCSVs of the SP drug solutions at pH lower than 7.0 displayed one reduction peak at −1.05 V ($E_{p,c1}$)H assigned to the reduction of the carbonyl group of pregn-4-ene-21-carboxylic acid, 7-(acetylthio)-17-hydroxy-3-oxo, γ-lactone (7α, 17α)-, aldactone to pregn-4-ene-21-carboxylic acid, 7-(acetylthio)-17-hydroxy-3-hydroxy-, γ-lactone (7α, 17α)-, aldactone in two electrons reduction step ($2H^+/2e$) [13] (Scheme 1). In solutions of $7 < pH < 10$, the DPCSVs of SP drug showed two peaks in the range from −1.11 to −1.20 V (peak I, $E_{p,c1}$) and from −1.36 to −1.46 V (peak II, $E_{p,c2}$) and were safely assigned to successive reduction of the SP drug in two consecutive reduction steps (H^+/e) [18,19]. On increasing the solution pH ($7 < pH < 10.5$), the values of $E_{p,c1}$ and $E_{p,c2}$ were shifted cathodically and the plot of pH vs. $E_{p,c1}$or $E_{p,c2}$ was linear confirming direct exchange of one H^+/one e^- in two successive single-electrochemical steps leading to the conversion of C═O group to –CH–OH group [16]. At $pH > 10.5$, peak I disappeared and peak II was ill defined and affected by adsorption.

The dependence of CV of SP drug at HMDE on pH was critically investigated. Representative CV at pH 9 at 200 mV/s scan rate at HMDE vs. Ag/AgCl electrode is shown in Fig. 2.

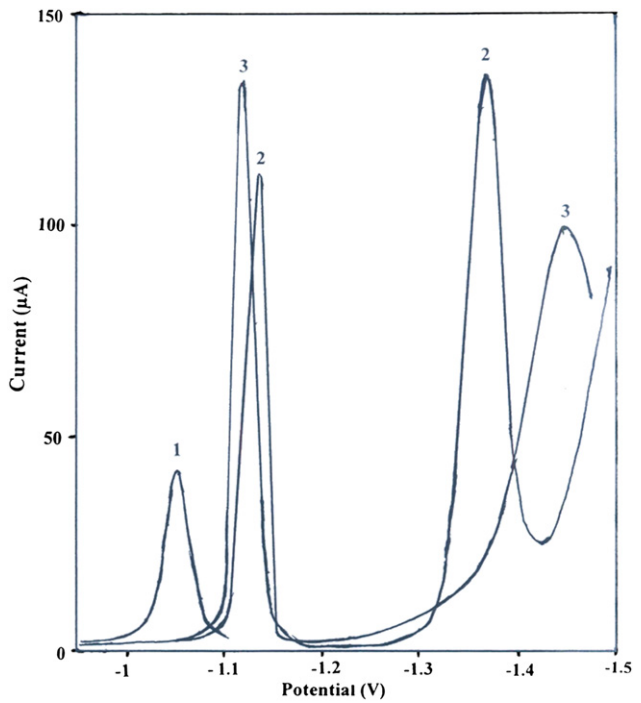


Fig. 1 DPCSVs of SP (4.8×10^{-6} M) at pH 3(1), pH 9 (2) and pH 7 (3) at the HMDE vs. Ag/AgCl reference electrode. $E_{acc} = -0.25$ V; $t_{acc} = 100$ s; scan rate = 60 mV/s and pulse amplitude of −50 mV.

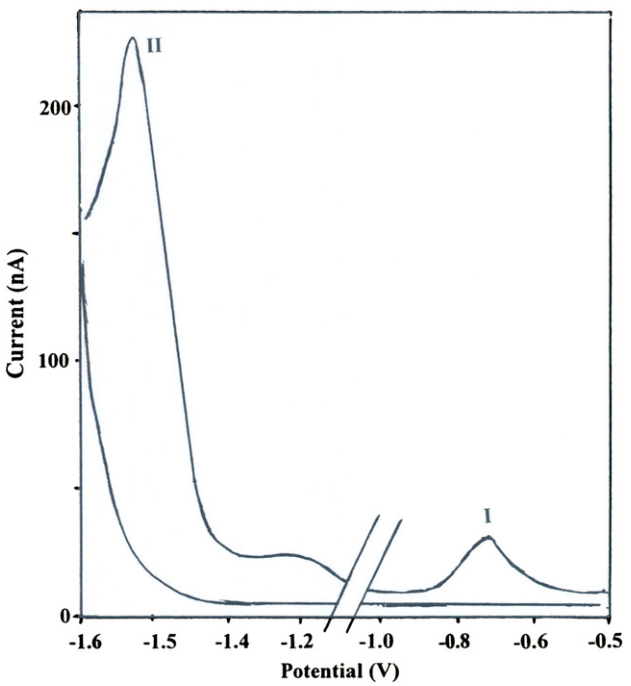
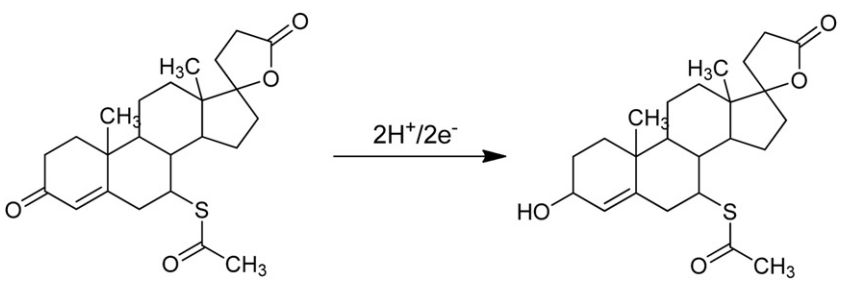


Fig. 2 CVs of SP (1.2×10^{-4} M) at pH 9.0 at HMDE vs. Ag/AgCl electrode at 200 mV/s scan rate.



Scheme 1

In solutions of pH < 7, one reduction peak was observed in the range from −1.38 to −1.42 V, while in solutions of 7 < pH < 10.3, two cathodic peaks were noticed. On raising the solution pH (7 < pH < 10.5), the potential of $E_{p,c1}$ or $E_{p,c2}$ of SP drug at 200 mV/s was shifted to more negative potential confirming the irreversible nature of the process and the electrode reaction involves hydrogen ions [18,19]. On the reverse scan, no anodic peaks were noticed confirming the irreversible nature of the process.

The influence of the scan rate, ν (50−2000 mV/s) on the CV of SP at pH 9 was studied at HMDE. Two peaks I and II were observed and were assigned to two H^+/e^- consecutive reduction steps of C = O to –CH–OH group. No anodic peaks were noticed on the reverse scan indicating the irreversible nature of the reduction process [19]. On increasing the scan rate, $E_{p,c1}$ and E_p,c_2 at pH 7–9 were shifted cathodically confirming the irreversible nature of the reduction steps [19]. The plots of $I_{p,c2}$ vs. v increased linearly confirming the adsorption process of SP at HMDE [19].

The variation of the current function ($I_{p,c}/v^{1/2}$) with scan rate is an important diagnostic criterion for distinguishing between the ECE (chemical reaction coupled between two charge -transfer processes) and EE (two successive one-electron charge-transfer processes) [19] type mechanisms. The plot of $I_{p,c}/v^{1/2}$ vs. v of peak I increased linearly on raising the v indicating that the observed behavior does not favor the EC mechanism. This finding may be taken as an indication of the CE mechanism with an irreversible reduction step of the drug [19,20]. The observed behavior may possibly be explained by considering that the protonation reaction is very fast or virtually complete, so that, the electrode reaction appears to be of the ECE type at the scan rates used in the present investigation. In the CV, a small reduction peak current at high v corresponding to peak I was noticed. Thus, it can be concluded that, the reduction step undergoes a very rapid follow-up chemical reaction [13,14]. The dependence of the CV response on analyte concentration at pH 9.0 showed no significant changes on the cathodic current of the observed cathodic peak at −1.20 V, indicating that the electrochemical process is a typical of ECE type electrochemical mechanism [19].

The value of the electron transfer coefficient (α) involved in the rate determining step was calculated employing the following equation [21]:

$$\Delta E/\Delta \log v = -29.58/\alpha n_\alpha \quad (1)$$

where n_α is the number of electron transfer in the rate determining step. Assuming n_α is equal 1 or 2, the computed values of α from the linear plots of linear plots of log v vs. $E_{p,c1}$ of peak I and log v vs. $E_{p,c2}$ of peak II were found higher than 0.6 confirming the irreversible nature of the observed reduction steps [13,20]. This trend is also indicative of kinetic complications in the electrode process and the reduction process comprises several reactions including adsorption [13,19].

The surface coverage (Γ) of the electroactive species was calculated from the CVs using the following equation [21]:

$$I_{p,c} = n^2F^2A\Gamma\upsilon/4RT \quad (2)$$

where, n= number of electron, A = area of the electrode surface, cm^2, T is the absolute temperature and R is the gas constant. For n = 2, a value of Γ was found equal 77×10^{-6} M encouraging application of the DPCSV for SP determination.

3.2. Analytical parameters

Preliminary DPCSV investigation has shown that, the current of peak I was independent from the SP concentration; however, the $I_{p,c2}$ of peak II is dependent. The high degree of surface coverage of SP onto the HMDE and sensitivity of cathodic peak II towards SP concentration encouraged studying the analytical parameters that control the peak current of peak II using DPCSV procedures. Thus, peak I was not selected in the next work, while the influence of analytical parameters that control cathodic peak current of peak II was critically studied. The plot of pH vs. $I_{p,c2}$ (Fig. 1) revealed that, $I_{p,c2}$ reached the maximum at pH 9–10.3 and in this pH range, the cathodic peak was sharp and symmetric. Thus, in the subsequent work, the solution pH was adopted at pH 9–10.

The effect of the adsorption time (t_{ad}) on the collection and stripping procedure of SP drug at the HMDE was tested in the range of 30–180 s. The maximum peak current was achieved at t_{ad} of 60 s (Fig. 3) for SP solution (5×10^{-7} M). At accumulation time greater t_{ad} than 180 s, the peak current leveled off because of adsorption saturation of SP drug at the HMDE (Fig. 3). Because of the strong adsorption of SP drug at the surface of the HMDE at the equilibrium time, the plot of t_{ad} vs. $I_{p,c}$ of peak II did not passed through the origin [19,20].

The effect of deposition potential ($E_{ad.}$) on the peak current of peak II (−1.38 V) at HMDE vs. Ag/AgCl reference electrode was studied. The maximum peak current was achieved at E_{ad} = −0.45 V. At deposition potential < −0.45 V, the background current gradually deteriorates (Fig. 4). Thus, a deposition potential of −0.45 V was selected in the next work. The effect of v (10−60 mV/s) at pH 9–10 on the I_p,c_2 of peak II at the HMDE was tested at the optimum t_{ad} and E_{ad}. At scan rate of 60 mV/s, the $I_{p,c2}$ increased steadily on raising the v and best background, sensitivity and peak resolution were achieved. Thus, a 60 mV/s scan rate was adopted in the next work.

The effect of pulse amplitude (−90 to 100 mV) on the DPCSV peak under the optimal conditions was studied. The peak current increased steadily on decreasing pulse amplitude

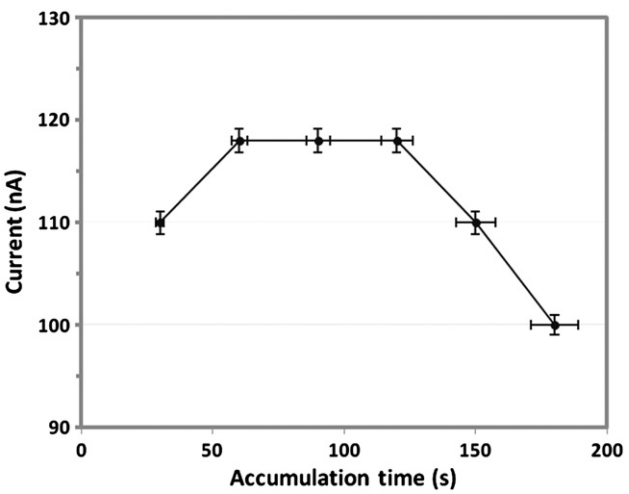


Fig. 3 Influence of deposition time on the $I_{p,c2}$ of SP (4.8×10^{-7} M) at pH 9 at HMDE vs. Ag/AgCl electrode.

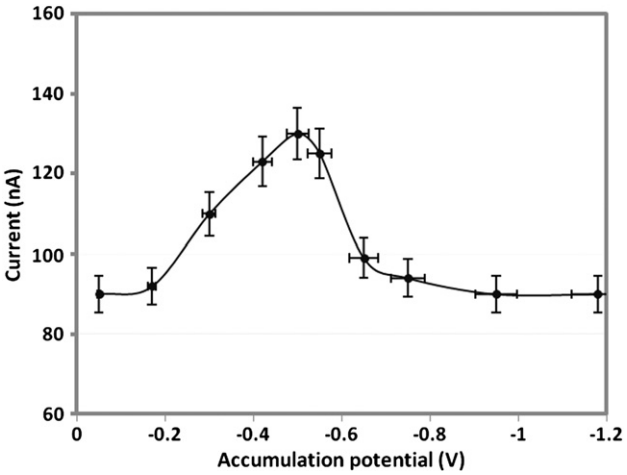


Fig. 4 Influence of deposition potential on the $I_{p,c2}$ of SP (4.8×10^{-7} M) at pH 9 at HMDE vs. Ag/AgCl electrode.

down to −50 mV. At this value, best sensitivity and peak current were achieved. Thus, in the next work a pulse amplitude of −50 mV was chosen. The influence of varying the starting potential (0.0 to −1.0 V) on the DPCSV peak current at 1.40 V was evaluated at HMDE. At starting potential <0.0 V, the value of $I_{p,c2}$ decreased due to the prior reduction of SP drug at a starting potential close to −0.2 V. The maximum peak current was achieved at 0.0 V, hence, a starting potential of 0.0 V was selected in the next work.

3.3. *Figure of merits*

Under the optimum experimental conditions of pH 9–10, deposition time 60 s, deposition potential −0.45 V, pulse amplitude −50 mV, starting potential −0.0 V and scan rate 60 mV/s, the DP CSV of SP showed that, the $I_{p,c1}$ at −1.1 V was not sensitive and independent on SP concentration, while the $I_{p,c2}$ at −1.40 V vs. Ag/AgCl increased linearly on increasing the drug concentration in the range from 1.2×10^{-10} to 9.6×10^{-7} M (Fig. 5). Above 9.6×10^{-7} M, the $I_{p,c2}$ tended to level off because of the adsorption saturation with the following a regression equation:

$$I_p c_2(\text{nA}) = 1.71C(\mu M) + 3E^{-14} \left(R^2 = 0.995\right)$$

According to International Union of Pure and Applied Chemistry (IUPAC) [22], the lower limit of detection (LLOD= $3S_{y/x}/b$) and limit of quantification (LOQ=$10S_{y/x}/b$), where $S_{y/x}$ is the standard deviation of y-residual and b is the slope of the calibration plot of SP, were found equal 1.1×10^{-11} and 4.14×10^{-11} M. A relative standard deviation (RSD) of SP at 8.5×10^{-7} M was found equal 2.39% (n=5). The main analytical features of the proposed method were compared with the reported CdSe quantum dots as luminescent probes [9], polarographic [13] and square wave DP CSV [14] methods for SP determination. The figures of merits (linear dynamic range, LLOD, LOQ and RSD) of the developed DPCSV method are better than those of the HPLC [7], polarographic [13], SW-AdSV [14] and official [23] methods. Although the present method requires higher overvoltage (−1.4 V) which is more liable to be interfered by other redox species than the SW-AdSV (−1.0 V) [14], the present method is selective, rapid, and shows excellent figures of merits.

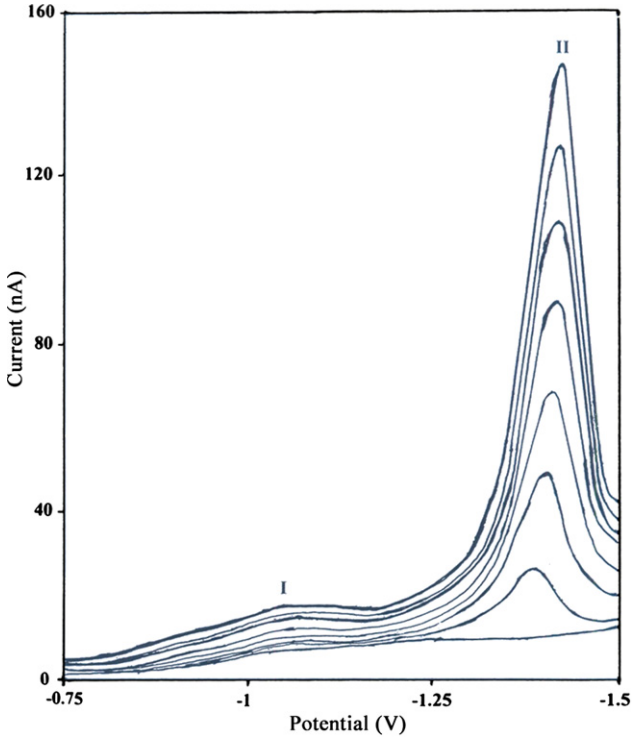


Fig. 5 DPCSVs of SP in BR buffer of pH 9 at HMDE vs. Ag/AgCl reference electrode at various SP concentrations. Conditions: E_{acc}=−0.45 V, t_{acc} =60 s, v=60 mV/s, pulse amplitude −50 mV and 0.0 V starting potential.

3.4. *Selectivity*

The selectivity of the DPCSV method was estimated by adding various excipients, diluents and active ingredients e.g. magnesium stearate, talcum powder, sodium lauryl sulfate, sucrose, glucose, lactose maltose, starch and mannitol used in pharmaceutical formulations. Each excipient (0.4–0.6 g) was added according to the manufacturer's batch formula to known concentration of SP (9.6×10^{-7} M). The tolerable limit was defined as the concentration of the excipient causing a deviation in the range ±3.0% of the peak current at −1.45 V vs. Ag/AgCl of SP solution under the optimum condition. No significant changes on the magnitude of $I_{p,c2}$ (nA) by more than ±3.0% were noticed. Thus, the method is free from the tested excipients. A series of SP solutions ($9.6.0 \times 10^{-7}$M) containing atenolol, metoprolol, amiloride, aspirin, or quinine individually at concentration of 9.6×10^{-7} M were also tested. Amiloride drug interfered seriously at a concentration of amiloride 100 times SP. This behavior is most likely attributed to the competitive adsorbability of amiloride with SP drug on the surface of the HMDE at the optimum pH.

3.5. *Analytical application*

3.5.1. *Analysis of SP in pure- and dosage form*

The method was applied to the analysis of SP in pure form and in pharmaceutical preparations via calibration plot and

Table1 Determination of SP ($n=3$) in pure form and in dosage form (Aldactone, 25 mg/tablet) by direct calibration (A) and the standard addition (B) of the DPCSV method, HPLC (C) and the official titrimetric (D) procedures.[a]

Drug product	A	B	C	D
Pure drug (%)	97.4±2.2	101.4±2.4	97.9 ±1.7	98.4±2.9
RSD(%)	2.56	2.1	2.3	3.1
t-Value[a]			1.63 (2.31)	1.54 (2.31)
F-value[b]			1.29 (2.31)	1.75 (2.31)
			2.20 (6.39)	2.7 (6.39)
			1.18 (6.39)	2.64 (6.39)
Aldactone tablet	97.9±2.4	101.2±1.9	98.5±1.7	98.5±1.7
RSD	–	±3.66	±2.93	±3.85

[a]Average recovery$\pm ts/n^{1/2}$; $n=3$.
[b]The theoretical figures of *t*- and *F*-values at $P=0.05$ are given in parentheses.

standard addition method. The results are summarized in Table 1. These results were validated by comparison with the standard HPLC [7] and the official methods [23] data following the method of validation [24]. A recovery of 97.35±2.2%, with RSD of ±2.56 in pure drug was achieved via direct linear plot in good agreement with the results obtained via the standard addition procedure (101.4±2.4%, RSD=±2.10). These results were successfully validated by comparison with standard HPLC (97.9±1.7%, RSD=±2.3) and the official (98.4±2.9%, RSD=±3.1) [7,23,24] methods. The *F* and student *t*- tests showed no significant difference between the developed and these methods [7,23–25].

The results of analysis of SP in Aldactone (Searle) tablet (25.0 mg/tablet) by the developed method via calibration plot and standard addition are given in Table 1. The results of the present method (24.46±0.9 mg/tablet, $n=3$) are in agreement with the claimed value (25.0 mg/tablet), HPLC (24.6±0.72 mg/tablet) [7] and the official (25.2±0.97 mg/tablet) [23,24] methods. At the 95% probability, the Student's *t*- and *F* tests showed no significant differences between the developed DPCSV, HPLC [7] and the official [23,24] methods (Table 1).

3.5.2. *Analysis of drug in wastewater*

The sensitivity of the developed DP CSV encourages determination SP residues in tap and industrial wastewater samples by the spiking method. Various volumes (10–50 μL) of standard SP (0.3 μg/mL in ethanol) were added to wastewater sample and analyzed as described in the experimental section. The recovery percentage of the developed method (101.80±2.9%) was close to the results obtained by the official titrimetric method (98.6±2.7%) [23,24] for SP drug added. The *t*- (1.78) and *F*- (1.25) tests at 99% confidence levels did not exceed the theoretical ones 2.31 and 6.39, respectively, confirming the accuracy of the DP CSV method.

4. Conclusion

The developed method provides an excellent alternative approach for the determination of SP in comparison with HPLC, polarographic and voltammetric and the official methods. The sensitivity and selectivity of the developed procedures for the determination of SP in various matrices could be improved by the preconcentration from large sample volumes onto solid sorbent packed column followed by elution and subsequent analysis. Work is continuing for the application of on-line stripping analysis of SP in serum, blood and environmental samples.

Acknowledgments

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References

[1] I. Ali, V.K. Gupta, P. Singh, et al., Screening of domperidone in wastewater by high performance liquid chromatography and solid phase extraction methods, Talanta 68 (2006) 928–931.
[2] K. Kummerer, Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources: A review, Chemosphere 45 (2001) 957–969.
[3] M. Ouzounian, A. Hassan, J.L. Cox, et al., The effect of spironolactone use on heart failure mortality: a population-based study, J. Card. Failure 13 (2007) 165–169.
[4] J.J. Bradstreet, S. Smith, D. Granpeesheh, et al., Spironolactone might be a desirable immunologic and hormonal intervention in autism spectrum disorders, Med. Hypotheses 68 (2007) 979–987.
[5] C.S. Rigsby, D.M. Pollock, A.M. Dorrance, Spironolactone improves structure and increases tone in the cerebral vasculature of male spontaneously hypertensive stroke-prone rats, Microvasc. Res. 73 (2007) 198–205.
[6] P.K. Singh, D.P. Singh, Effect of spironolactone on acid and alkaline phosphatase in the testes of albino rat, Indian J. Clin. Biochem. 20 (1) (2005) 115–116.
[7] A. Jankowski, A.S. Jankowska, H. Lamparczyk, Simultaneous determination of spironolactone and its metabolites in human plasma, J. Pharm. Biomed. Anal. 14 (1996) 1359–1365.
[8] R. Herraezhernandez, E. Sorianovega, P. Campinsfalco, High-performance liquid chromatographic determination of spironolactone and its major metabolite canrenone in urine using ultraviolet detection and column-switching, J. Chromatogr. B Biomed. Appl. 658 (1994) 303–310.

[9] J.G. Liang, S. Huang, D.Y. Zeng, et al., CdSe quantum dots as luminescent probes for spironolactone determination, Talanta 69 (2006) 126–130.
[10] O. Hernandez, E. Martin, F. Jimenez, et al., Use of partial least-squares regression for multicomponent determinations based on kinetic spectrofluorimetric data. Simultaneous determination of canrenone and spironolactone in urine, Analyst 125 (2000) 1159–1165.
[11] M.L. Luis, J.M. Garcia, F. Jimenez, et al., Simultaneous determination of chlorthalidone and spironolactone with univariate and multivariate calibration: wavelength range selection, J. AOAC Int. 82 (1999) 1054–1063.
[12] E. Martin, O. Hernandez, A.I. Jimenez, et al., A partial least-squares multivariate calibration method for the simultaneous spectrophotometric determination of spironolactone, canrenone and hydrochlorothiazide, Anal. Lett. 31 (1998) 1857–1877.
[13] F. Belal, Polarographic behaviour and determination of spironolactone, Acta Microchim. 107 (1992) 11–17.
[14] A.H. Al-Ghamdi, A.F. Al-Ghamdi, M.A. Al-Omar, Electrochemical studies and square-wave adsorptive stripping voltammetry of spironolactone drug, Anal. Lett. 41 (1) (2008) 90–103.
[15] M.S. El-Shahawi, S.O. Bahaffi, T. El-Mogy, Analysis of domperidone in pharmaceutical formulations and wastewater by differential pulse voltammetry at a glassy-carbon electrode, Anal. Bioanal. Chem. 387 (2007) 719–725.
[16] E. Fischer, C.M.G. Van Den Berg, Determination of lead complexation in lake water by cathodic stripping voltammetry and ligand competition, Anal. Chim. Acta 432 (1) (2001) 11–20.
[17] K. Yokio, A. Yamaguchi, M. Mizumachi, et al., Direct determination of trace concentrations of lead in fresh wáter samples by adsorptive cathodic stripping voltammetry of a lead–Calcein Blue complex, Anal. Chim. Acta 316 (3) (1995) 363–369.
[18] N. Abo-el-Maali, Voltammetric analysis of drugs, Bioelectrochemistry 64 (2004) 99–107.
[19] A.J. Bard, L.R. Faulkner, Electrochemical Methods: Fundamentals, John Wiley & Sons, New York, 1980 218.
[20] Z. Galus, Fundamentals of Electrochemical Analysis, Ellis Harwood, Ltd., New York, 1976.
[21] D.T. Sawyer, W.R. Heinemann, J.M. Beebe, Chemistry Experiments for Instrumental Methods, John Wiley &Sons, New York, 1984.
[22] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, 4th Ed., Ellis-Horwood, Limited, New York, 1994 115.
[23] U.S. Pharmacopeias, 27th Ed. US Convention, Rockville, MD, 2004.
[24] The British Pharmacopoeia, Her Majesty's Stationary Office, London, 1988, pp. 531, 1003.
[25] J. Ermer, J.H. McB.Miller, Method Validation in Pharmaceutical Analysis, 1st Ed., Wiley – VCH Pub., Germany, 2005.