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Voltammetry and quantification of the contraceptive drug norethisterone in bulk form and pharmaceutical formulation

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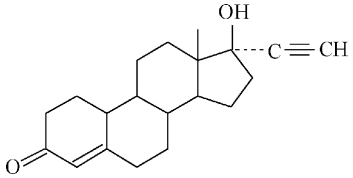
Abstract

The electrochemical behavior of norethisterone at the mercury electrode was studied in the universal buffer of various pH values using dc-polarography, cyclic voltammetry and controlled-potential electrolysis. Norethisterone was reduced at the mercury electrode via the consumption of two electrons corresponding to reduction of the 3-keto-delta-4-group in the A-ring of the molecule. The pK_a value (8.7) of norethisterone was determined from the polarographic and spectrophotometric measurements. A fully validated, simple, sensitive, precise and inexpensive square-wave adsorptive cathodic stripping (SWAdCS) voltammetry procedure was described for trace quantification of bulk norethisterone. The stripping voltammetry peak current of norethisterone in a universal buffer of pH 5 following its accumulation onto the hanging mercury drop electrode (HMDE) at −0.6 V (versus Ag/AgCl/KCl$_s$) for 130 s showed a linear response with the concentration over the range 5×10^{-9} to 3×10^{-7} M norethisterone. Detection and quantitation limits of 1.5×10^{-9} and 5×10^{-9} M bulk norethisterone, respectively, were achieved. The proposed procedure was successfully applied for the assay of norethisterone in Steronate tablets without interference from excipients.

Keywords: Norethisterone; Polarography; Cyclic voltammetry; Square-wave adsorptive stripping voltammetry; Quantification; Steronate tablets

1. Introduction

Norethisterone (17-alpha-ethinyl-17 beta-hydroxy-4-estren-3-on) is a synthetic progestagenic compound widely used in gynecological practice for contraception and hormone replacement therapy in postmenopausal women. It acts to inhibit the secretion of pituitary gonadotrophins that prevents follicular maturation and ovulation [1].



(Structure of norethisterone molecule)

Various analytical methods have been described for assay of norethisterone in bulk form, pharmaceuticals and biological fluids. These mainly include: high-performance liquid chromatography [2–7], capillary gas chromatography [8–14], radioimmunological and chromato-mass fragmentography [1], argentometry [15] and differential-pulse polarography [16–19]. Adsorptive cathodic stripping voltammetry has been shown as an efficient analytical technique for trace determination of a wide range of drugs, which have an interfacial adsorptive character onto surface of the working electrode. However, to date no stripping voltammetric procedure is reported in literature for quantification of norethisterone.

The aim of this study was to clarify the electrochemical behavior of norethisterone at the mercury electrode and to describe a simple and sensitive square-wave adsorptive cathodic stripping (SWAdCS) voltammetry procedure for its quantification in bulk form and in the pharmaceutical formulation (Steronate tablets).

2. Experimental

2.1. Instrumentation

A polarograph (Model 4001 Sargent-Welch) was used for the polarographic measurements. A polarographic cell with a drop-

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ping mercury electrode as a working electrode ($m = 1.05$ mg s^{-1}, $t = 3.3$ s at mercury height = 60 cm) and a saturated calomel electrode (SCE) as a reference electrode was used. A computer-controlled Electrochemical Analyzers Models 263A and 394-PAR (Princeton Applied Research, Oak Ridge, TN, USA,) with the software package 270/250-PAR were used for the voltammetric measurements. An electrode assembly (303A-PAR) incorporated with a micro-electrolysis cell and a three-electrode configuration system comprising of a hanging mercury drop electrode (HMDE) as a working electrode (surface area = 0.026 cm^2), an Ag/AgCl/KCl$_s$ reference electrode and a platinum wire auxiliary electrode was used. A magnetic stirrer (305-PAR) and a stirring bar were used to provide the convective transport of the analyte during its accumulation onto the mercury electrode surface.

A potentiostat/galvanostat (Model 173-PAR) incorporated with a digital coulometer Model 179-PAR was used for controlled-potential electrolysis. A micro-coulometric cell incorporated with a mercury pool as a working electrode, a saturated calomel electrode as a reference electrode and a platinum gauze as a counter electrode was used. The potential applied during the potential-controlled electrolysis was adjusted to be equal to the $E_{1/2}$ of the polarographic wave of norethisterone at the studied pH plus −0.1 V. The total charge (Q) passed during the exhaustive electrolysis of norethisterone was obtained by integrating the current electronically. Using Faraday's equation: $N = Q/nF$ (where N is the number of moles of substance being electrolyzed), the number of electrons (n) transferred per norethisterone molecule was determined and was found to be equal to two, which attributed to reduction of the 3-keto-delta-4-group in the A-ring of the analyte molecule.

A Shimadzu UV–vis recording Spectrophotometer (Model 160A) was used for the spectral study of norethisterone in buffered solutions.

2.2. Materials and solutions

2.2.1. Norethisterone solutions

Authentic norethisterone was kindly supplied from Schering AG (Berlin, Germany). A stock standard solution of 1×10^{-3} M bulk norethisterone was prepared in ethanol (Merck), and then stored at 4 °C. Working solutions of norethisterone (10^{-6} to 10^{-4} M) were prepared daily by appropriate dilution with ethanol just before use.

2.2.2. Tablet solutions

Twenty tablets of Steronate (Norethisterone acetate; NETA, labeled as containing 5 mg NETA per tablet—HI Pharm., Cairo, Egypt) were weighed, and then the average mass per tablet was determined. The tablets were grounded to a homogeneous fine powder. A quantity of the homogeneous powder of NETA equivalent to the weight of two tablets was accurately transferred into a 100 ml volume calibrated flask contains 70 ml ethanol (Merck). The content of the flask was sonicated for about 10 min and then made up to the volume with ethanol. The solution was then filtered through a 0.45 μm milli-pore filter (Gelman, Germany). The desired concentrations of the NETA were obtained by accurate dilution with ethanol. The solution was directly analyzed according to the general analytical procedure. A Mettler balance (Toledo-AB104, Switzerland) was used for weighing the solid materials.

2.2.3. Supporting electrolyte

A series of the universal buffer of pH 2–11.5 as a supporting electrolyte was prepared [20]. A pH-meter (Crison, Barcelona, Spain) was used for the pH measurements. All the chemicals used (Merk) were of analytical-reagent grade and were used without further purification. The de-ionized water was supplied from a Purite-Still Plus de-ionizer connected to an AquaMatic double-distillation water system (Hamilton Laboratory Glass Ltd., Kent, UK).

2.2.4. General analytical procedure

A known volume of the standard stock solution of norethisterone was pipetted into a 10 ml volume calibrated flask and then made up to the volume with the universal buffer of pH 5. The solution was introduced into the electrolysis cell, and then deoxygenated with pure nitrogen gas for about 10 min in the first cycle and for 30 s in each successive cycle, while the nitrogen gas was kept over the solution during the measurements. Accumulation of norethisterone onto the surface of HMDE was performed at −0.7 V (versus Ag/AgCl/KCl$_s$) for 130 s while stirring the solution at 400 rpm. After an equilibrium time of 5 s allowed for the solution to become quiescent, the voltammograms were recorded by scanning the potential towards the negative direction using the square-wave waveform. A calibration graph was constructed under the optimized conditions of the procedure.

3. Results and discussion

3.1. Dc-polarography

The dc-polarograms of 1.25×10^{-4} M bulk norethisterone in the universal buffer of pH <7.7 showed a single 2-electron irreversible cathodic wave which may be attributed to reduction of the 3-keto-delta-4-group in the A-ring of the norethisterone molecule. On the increase of pH of the medium this wave splits into two waves, the height of the second wave increased at the expense of the first one until the latter disappeared completely in solutions of the pH values ≥9.5 (Fig. 1) at which the polarogram exhibited a single 2-electron wave at more negative potentials. The cumulative i_l–pH curve for the single wave and the first splitting one (Fig. 2, curve a) or that for the second splitting wave and the single wave (Fig. 2, curve b) recalled dissociation (*Z*-shaped) or association (*S*-shaped) curves, respectively, which may be attributed to presence of norethisterone in an acid–base equilibrium [21]. The two i_l–pH curves (Fig. 2, curves a and b) intersected at a pH value equals to the pK'_a of norethisterone (pH = pK_a = 8.7) which agrees well with the value (pK_a = 8.5) obtained from a preliminary spectrophotometric study of 3×10^{-5} M norethisterone in the universal buffer of various pH values (pH 3–10).

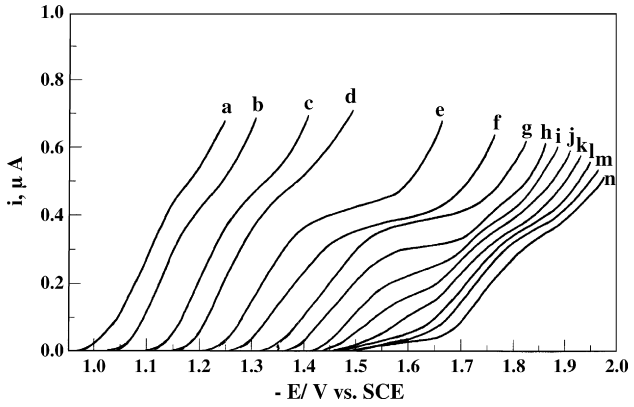

Fig. 1. Dc-polarograms for 1.25×10^{-4} M bulk norethisterone in the universal buffer of various pH values: (a) 2.3; (b) 2.7; (c) 3.3; (d) 4.3; (e) 5.3; (f) 5.9; (g) 6.7; (h) 7.7; (i) 8.5; (j) 8.8; (k) 9.2; (l) 10.0; (m) 10.5; and (n) pH 11.4.

The half-wave potentials ($E_{1/2}$), of the single wave recorded in solutions of pH <7.7, and that of the first splitting wave recorded in solutions of pH values 7.7–9.2 (Fig. 1), shifted toward more negative values with the increase of pH. This behavior indicated the involvement of protons in the rate-determining step and that the proton transfer precedes the electron transfer reaction [21]. On the other hand, the $E_{1/2}$ of both the second splitting wave (pH 7.7–9.2) and the single wave (pH ≥ 9.5) were practically pH-independent. The linear $E_{1/2}$–pH plot for both the single wave at pH values <7.7 and the second splitting one at pH values 7.7–9.2 and that of the single wave at pH values ≥9.5) intersected at a pH value equals to the pK_a value of norethisterone (pH = pK_a = 8.7) which agrees well with that value obtained from either the i_l–pH or the absorbance—pH curves.

Thus, sequence of the electrode reaction of the 3-keto-delta-4-group in the A-ring of norethisterone molecule at the mercury electrode can be expressed as follows [21]:

(i) In acidic and neutral solutions (pH < 7.7) the sequence is proton, electron, electron, proton; (ii) in alkaline solutions

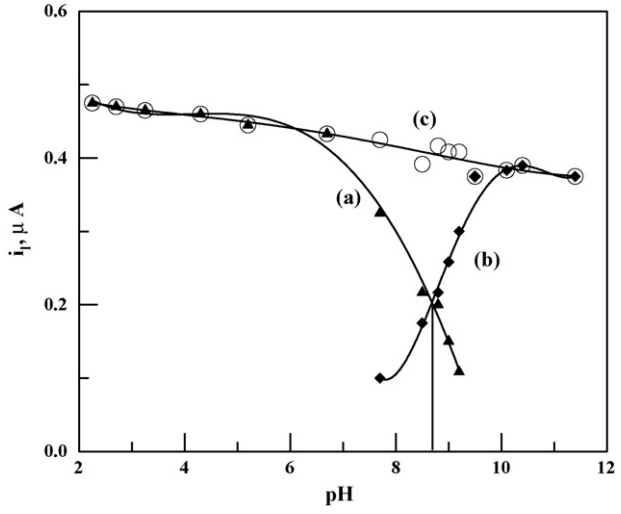

Fig. 2. i_l–pH plots for 1.25×10^{-4} M bulk norethisterone. (a) The single wave (pH < 7.7) and the first splitting wave (pH 7.7–9.2); (b) the second splitting wave (pH 7.7–9.2) and the single wave (pH ≥ 9.5); (c) total limiting current.

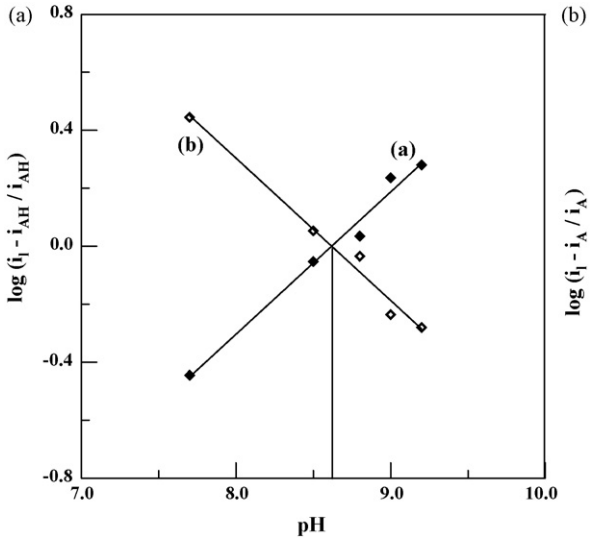

Fig. 3. Plots of $\log[(i_l - i_{HA})/i_{HA}]$ and $\log[(i_l - i_A^-)/i_A^-]$ vs. pH for 1.25×10^{-4} M bulk norethisterone: (a) first splitting wave; (b) second splitting wave recorded in solutions of pH 7.7–9.2.

of pH values ≥9.5 the sequence is electron, electron, proton, proton; and (iii) in solutions of intermediate pH values 7.7–9.2 norethisterone molecule is expected to present in an acid–base equilibrium (p$K'_a \approx 8.7$),

$$AH \underset{k_r}{\overset{k_d}{\rightleftharpoons}} A^- + H^+ \quad \left(K = \frac{k_d}{k_r}\right)$$

both the acidic (AH) and basic (A^-) forms are electro-active. Thus, the reduction of norethisterone over the pH range 7.7–9.2 takes place via two steps, and the limiting current i_l of the second step (reduction of A^- form) increased at the expense of that of the first one (reduction of AH form) on the increase of pH until the latter one disappeared completely in solutions of pH ≥9.5 (Figs. 1 and 2). Thus, the electrode reaction of norethisterone over the pH range (7.7–9.2) was considered to consist of mixed processes {(i) and (ii)}. According to Zuman [21], the existence of an electroactive compound in an acid–base equilibrium leads to the variation of the reduction current with the change of hydrogen ion concentration [H^+] as governed by the equation:

$$\frac{i_{AH}}{i_l} = \frac{0.886\{(k_r t_1/K)[H^+]\}^{1/2}}{1 + 0.886\{(k_r t_1/K)[H^+]\}^{1/2}}$$

which was modified to the following forms [21,22]:

$$pH = \log\ 0.886\left(\frac{k_r t_1}{K}\right)^{1/2} + \log\left(\frac{i_l - i_{AH}}{i_{AH}}\right)$$

$$pH = \log 0.886\left(\frac{k_r t_1}{K}\right)^{1/2} + \log\left(\frac{i_l - i_A^-}{i_A^-}\right)$$

where i_{AH} and i_A^- are the reduction currents of the acidic and basic forms, respectively. Plots of $[\log(i_l - i_{AH})/i_{AH}]$ or $[\log(i_l - i_A^-)/i_A^-]$ versus pH, respectively, for the first splitting or the second splitting waves were straight lines (Fig. 3) which

indicated the validity of the previous equations and consequently confirmed the presence of norethisterone in an acid–base equilibrium within the pH range 7.7–9.2. The pH value corresponds to the intersection point of the two curves (Fig. 3, curves a and b) equals to the pK'_a of norethisterone ($pH = pK'_a \approx 8.7$).

3.2. Cyclic voltammetry

Cyclic voltammograms of 1.25×10^{-4} M norethisterone at the HMDE in the universal buffer of pH values 2–5 exhibit a single 2-electron irreversible cathodic peak, which attributed to the reduction of the 3-keto-delta-4-group in the A-ring of the acidic form of norethisterone molecule. As the pH of the medium increased, the peak current intensity decreased and a new irreversible cathodic peak was observed at a more negative potential. Its peak current increased at the expense of the first one until the latter disappeared completely in solutions of the pH values ≥9.5. The single 2-electron cathodic peak observed at the pH values ≥9.5 may be attributed to the reduction of the 3-keto-delta-4-group in the A-ring of the basic form of norethisterone molecule.

The interfacial adsorptive character of norethisterone was identified by recording the cyclic voltammograms of 1×10^{-6} M in a universal buffer of pH 5 following accumulation of the drug onto the HMDE at open circuit (Fig. 4, curve a) and at −0.6 V for 60 s (Fig. 4, curve b) at scan rate 100 mV s^{-1}. The substantial enhancement of the peak current intensity of the first scan (curve b) compared to that of the subsequent scan at the same mercury drop (curve c) or with that recorded following accumulation of the drug at open circuit (curve a) confirmed the interfacial adsorptive character of norethisterone onto the mercury electrode. The adsorptive character of norethisterone was also identified by measuring the peak current (i_p) at various scan rates ν (50 − 500 mV s^{-1}) following accumulation onto the HMDE for 60 s at −0.6 V. A linear log i_p versus log ν plot following the equation: log i_p = 0.98, log ν–1.86, r = 0.999 was obtained. The slope value of 0.98 is close to the expected theoretical value 1.0 for an ideal reaction of surface species [23].

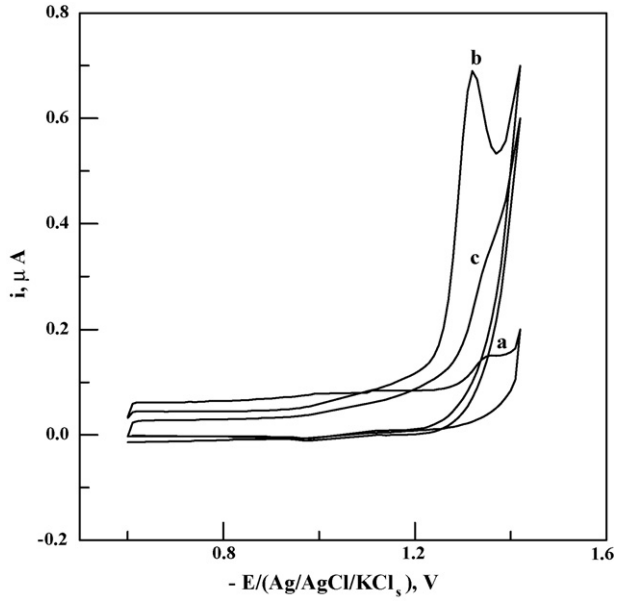

Fig. 4. Cyclic voltammograms for 1×10^{-6} M bulk norethisterone in a universal buffer (pH 5) at a scan rate = 100 mV s^{-1}, following accumulation onto the HMDE: (a) at open circuit; (b) at $E_{acc.}$ = −0.6 V for 60 s; and then the repetitive cycle at the same mercury drop (c).

The electrode surface coverage Γ_0 (amount of reactant adsorbed onto the mercury electrode surface, mol cm^{-2}) was calculated using the equation: $\Gamma_0 = Q/nFA$, where Q is the amount of charge consumed by the surface process as calculated by the integration of the area under the peak of the cyclic voltammogram of analyte corrected to residual current [24], n is the total number of electrons consumed in the reduction process (n = 2) and A is the mercury electrode surface area (0.026 cm^2). On dividing the number of coulombs transferred (1102 μC) by the conversion factor nFA (5017.3×10^9 μC), a surface coverage of 2.1964×10^{-10} mol cm^{-2} was obtained. Thus, each adsorbed norethisterone molecule occupies an area of 0.7559 nm^2.

3.3. Square-wave stripping voltammetry

3.3.1. Optimization of an analytical procedure

The influence of pH on square-wave adsorptive cathodic stripping (SWAdCS) voltammetric response for 5×10^{-7} M norethisterone was examined in the universal buffer of various pH values following accumulation onto the HMDE for 60 s at $E_{acc.}$ = −0.7 V. The voltammograms exhibited a single irreversible 2-electron cathodic peak, its intensity decreased markedly on the increase of pH of the medium. A well-defined peak current was achieved at pH 5. Therefore, a universal buffer of pH 5 was used as a supporting electrolyte in the rest of the present analytical studies.

The effect of instrumental conditions, e.g. frequency f (10–100 Hz), scan increment ΔE_s (2–10 mV) and pulse-amplitude a (10–50 mV) on the peak current intensity of 5×10^{-7} M norethisterone in the universal buffer (pH 5) following accumulation onto the HMDE for 60 s at $E_{acc.}$ = −0.7 V was studied. The achieved optimal instrumental conditions were f = 70 Hz, ΔE_s = 10 mV and, a = 25 mV.

The effect of accumulation potential ($E_{acc.}$) on SWAdCS voltammetry peak current intensity of 5×10^{-7} M norethisterone was examined over the range −0.3 to −0.9 V following accumulation onto the HMDE for 60 s. The peak current intensity was practically independent of the accumulation potential up to −0.6 V then decreased. Also effect of accumulation duration ($t_{acc.}$) at −0.6 V on the peak current intensity of various concentrations of bulk norethisterone (5×10^{-7}, 3×10^{-7}, 1×10^{-7}, 5×10^{-8} and 3×10^{-8} M) in the universal buffer of pH 5 was evaluated. As shown in Fig. 5, the response was linear up to 90 s for 5×10^{-7} M norethisterone solution and to 130 s for 3×10^{-7} and 1×10^{-7} M solutions, then leveled off (curves a, b and c). While for 5×10^{-8} and 3×10^{-8} M norethisterone solutions, as accumulation duration increases, linearity is prevailed over the tested accumulation durations (curves d and e). This means that the lower the concentration of the analyte, the longer of the accumulation duration is. Accordingly, the optimal accumulation conditions were $E_{acc.}$ = −0.6 V, and $t_{acc.}$ ≤130 s. The influence of the rest time was also considered and a time period of 5 s was chosen.

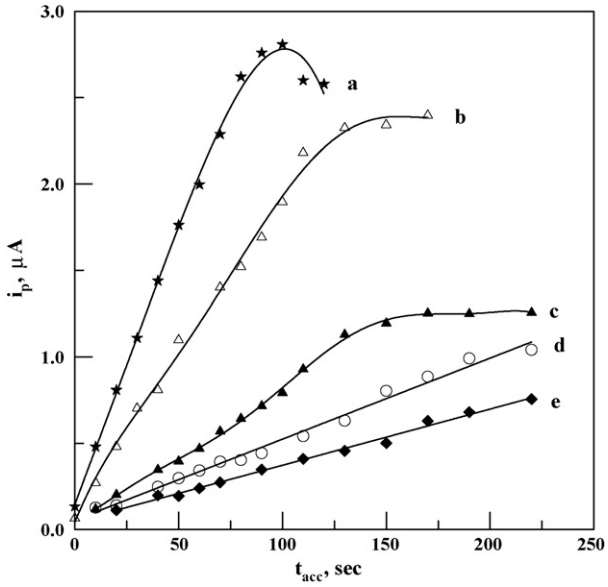

Fig. 5. Effect of accumulation duration ($t_{acc.}$), on the SWAdCS voltammetry peak current intensity for: (a) 5×10^{-7}; (b) 3×10^{-7}; (c) 1×10^{-7}; (d) 5×10^{-8}; and (e) 3×10^{-8} M norethisterone in a universal buffer (pH 5); $E_{acc.} = -0.6$ V; $f = 70$ Hz, $\Delta E_s = 10$ mV and $a = 25$ mV.

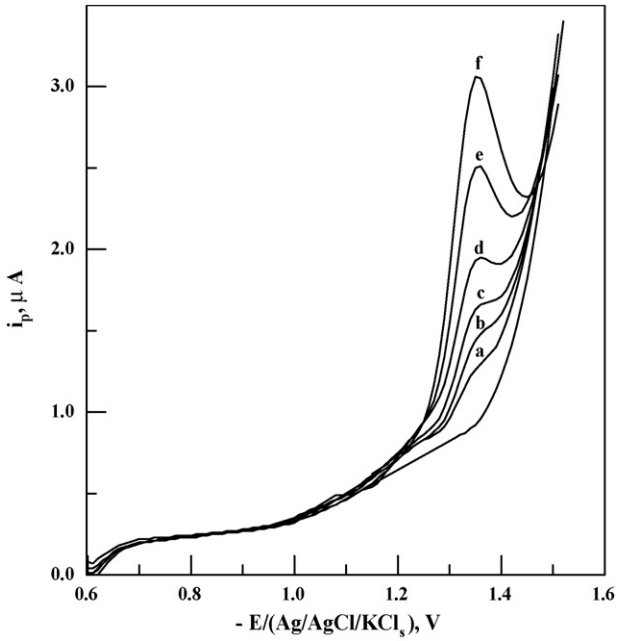

Fig. 6. SWAdCS voltammograms in a universal buffer of pH 5 for various concentrations of bulk norethisterone: the dotted line represents the background (a) 3×10^{-8}; (b) 5×10^{-8}; (c) 7×10^{-8}; (d) 1×10^{-7}; (e) 2×10^{-7}; and (f) 3×10^{-7} M; $E_{acc.} = -0.6$ V, $t_{acc.} = 130$ s, $f = 70$ Hz, $\Delta E_s = 10$ mV and $a = 25$ mV.

3.3.2. Procedure validation

Validation of the proposed procedure for assay of bulk norethisterone was examined via evaluation of limit of detection (LOD), limit of quantitation (LOQ), repeatability, reproducibility, precision, selectivity, robustness and intermediate precision [25]. As shown in Fig. 6, the stripping voltammetry peak current increased with concentration of norethisterone. Linear calibration graphs over various concentration ranges between 5×10^{-9} and 8×10^{-7} M bulk norethisterone, at various accumulation duration (40–130 s) were obtained. Characteristics of these calibration graphs are reported in Table 1. The LOD and LOQ of norethisterone were estimated at different accumulation durations, using the relation k S.D./b [26], where $k = 3$ for LOD and 10 for LOQ, S.D. is the standard deviation of the blank (or the intercept of the calibration curve) and b is the slope of the calibration graph. The results reported in Table 1, indicated the reliability of the proposed SWAdCS voltammetric procedure for the trace assay of bulk norethisterone.

The repeatability, reproducibility, precision and accuracy [25] of analysis using the proposed procedure were identified by performing three replicate measurements for 1×10^{-7} and 3×10^{-7} M bulk norethisterone ($t_{acc.} = 130$ s and $E_{acc.} = -0.6$ V) over one day (Intra-day assay) and for three days over a period of one week (inter-day assay). Satisfactory mean percentage recoveries (% R) and relative standard deviations (R.S.D.%) were achieved (Table 2).

The selectivity [25] of the optimized procedure was tested by analysis of a 1×10^{-7} M bulk norethisterone and a standard tablet solution containing 1×10^{-7} M norethisterone, following accumulation onto the HMDE for 130 s in both cases. No significant differences in the recoveries or the relative standard deviations were observed in the absence (100.20 ± 0.84) and presence (101.11 ± 1.32) of excipients. Thus, the proposed procedure can be considered selective.

Evaluating the influence of small variations in some of the most important procedural conditions, including pH (5–5.5), accumulation potential $E_{acc.}$ (−0.5 to −0.6 V) and accumulation duration $t_{acc.}$ (120–130 s) on the recovery of the drug, examined the robustness [25] of the procedure. The results showed that none of these variables significantly affect the recovery of norethisterone and provide an indication of the reliability of the proposed procedure for its assay. Thus, the proposed procedure could be considered robust. Moreover, the intermediate precision [25] of the measurements was examined by assay of bulk norethisterone using two electrochemical analyzers models, 263 A-PAR, Lab. (1) and 394-PAR, Lab. (2) under the same

Table 1
Characteristics of the calibration curves of SWAdCS voltammetric determination of bulk norethisterone; pH 5, $E_{acc.} = -0.6$ V, $f = 70$ Hz, $\Delta E_s = 10$ mV and $a = 25$ mV

$t_{acc.}$ (s)	Linearity range (M)	Regression equation i_p (μA) = bC (μM) + a	S_B	(r)	LOD (M)	LOQ (M)
40	2×10^{-8} to 8×10^{-7}	$i_p = 2.820\,C + 0.063$	5.64×10^{-3}	0.996	6.0×10^{-9}	2.0×10^{-8}
90	8×10^{-9} to 5×10^{-7}	$i_p = 5.144\,C + 0.175$	3.99×10^{-3}	0.999	2.3×10^{-9}	7.7×10^{-9}
130	5×10^{-9} to 3×10^{-7}	$i_p = 6.831\,C + 0.297$	3.42×10^{-3}	0.996	1.5×10^{-9}	5.0×10^{-9}

Table 2
Precision and accuracy of assay of norethisterone by the proposed SWAdCS voltammetry procedure ($n = 3$); $t_{acc.} = 130$ s

Conc. (taken) (M) $\times 10^7$	Conc. (found) (M) $\times 10^7$	R (%)	S.D.	Accuracy bias (%)	Precision % R.S.D.
Intra-day					
1	1.002	100.20	0.008	0.2	0.84
3	3.012	100.40	0.055	0.4	1.84
Inter-day					
1	1.014	101.4	0.016	1.40	1.60
3	3.037	101.23	0.069	1.23	2.87

Table 3
Assay of Steronate tablets by proposed SWAdCS voltammetry procedure ($t_{acc.} = 70$ s, $n = 4$) and a reported HPLC method [4]

Claimed (mg/tablet)	5.0
Recovery by the proposed method ($R\% \pm$ R.S.D.) (calibration curve method)	100.15 ± 1.42
Recovery by the proposed method ($R\% \pm$ R.S.D.) (standard addition method)	100.40 ± 1.59
Recovery by the reported HPLC method ($R\% \pm$ R.S.D.) (calibration curve method)	100.60 ± 1.85
F-value	1.697
t-test	0.386

The theoretical values of F- and t-test at 95% confidence limit (for $n_1 = 4$ and $n_2 = 4$) are 9.28 and 2.776, respectively.

procedural conditions at different elapsed times by two different analysts. The mean percentage recoveries obtained due to Lab. (1) to Lab. (2) and even day to day were found reproducible, since there is no significant difference between the recovery or relative standard deviation values.

3.3.3. Analysis of Steronate tablets

The proposed SWAdCS voltammetric procedure was successfully applied to assay of norethisterone in Steronate tablets without the necessity for samples pretreatment or time-consuming extraction steps prior to the analysis. The obtained results using calibration curve and standard addition [27] methods demonstrated the validity of the proposed procedure for assay of norethisterone in Steronate tablets (Table 3). The analysis exhibited satisfactory results, which were statistically compared with those obtained by a reported HPLC method [4]. Since the calculated F-value (Table 3) did not exceed the theoretical one, there was no significant difference between the proposed and reported methods with respect to reproducibility [28]. Also, no significant difference was noticed between the two methods regarding accuracy and precision as revealed by t-test [28], Table 3.

4. Conclusion

The electrochemical behavior of norethisterone at the mercury electrode was studied and discussed. Its pK_a was estimated from the polarographic and spectrophotometric measurements. A validated square-wave adsorptive cathodic stripping voltammetry procedure was described for trace quantification of norethisterone in its pharmaceutical formulation (Steronate tablets) The procedure is simple, selective, and precise.

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