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Voltammetric behavior and assay of the contraceptive drug levonorgestrel in bulk, tablets, and human serum at a mercury electrode

M.M. Ghoneim [a,*], W. Baumann [b], E. Hammam [a], A. Tawfik [a]

[a] *Department of Chemistry, Faculty of Science, Tanta University, 31527 Tanta, Egypt*
[b] *Institute of Physical Chemistry, University of Mainz, 55099 Mainz, Germany*

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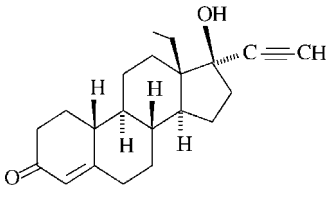
Abstract

The cyclic voltammograms of levonorgestrel (LNG) in Britton–Robinson buffers of pH 2–11 at the hanging mercury drop electrode showed a single two-electron irreversible cathodic peak over the whole pH range. This peak may be attributed to the reduction of the 3-keto-delta-4-group in the A-ring of the LNG molecule. The interfacial adsorptive character of levonorgestrel onto the surface of the hanging mercury drop electrode was identified by means of both cyclic voltammetry and chronocoulometry techniques. A simple, sensitive, and selective square-wave adsorptive cathodic stripping voltammetric procedure was developed for the quantitation of levonorgestrel. Under the optimized operational conditions, the maximum developed stripping voltammetric peak current showed a linear response with concentration of the bulk LNG substance. The achieved limits of detection (LOD) and quantitation (LOQ) were 6.7×10^{-10} and 2.2×10^{-9} M, or 4.8×10^{-10} and 1.6×10^{-9} M, following accumulation onto the hanging mercury drop electrode for 90 s or 150 s, respectively. The developed procedure was successfully applied to the determination of levonorgestrel in tablets, in spiked human serum, and in real plasma samples of healthy female volunteers following an oral administration of a 30-μg LNG single dose. The pharmacokinetic parameters ($C_{max} = 1.05\,ng\,ml^{-1}$, $t_{max} = 2.4\,h$ and $AUC_{0-t} = 16.5\,ng\,h\,ml^{-1}$) were estimated and favorably compared to those reported in literature for equivalent dose.

Keywords: Levonorgestrel; Cyclic voltammetry; Square-wave adsorptive stripping voltammetry; Assay; Pharmaceutical formulation; Microlut®; Human serum

1. Introduction

Levonorgestrel (LNG): (−)-13-ethyl-17-hydroxy-18,19-dinor-17 alpha-pregn-4-en-20-yn-3-one, is a synthetic progestin used as a progestin-only emergency contraceptive, and when administered at lower doses either alone or in combination with an estrogen, as an oral contraceptive.



Structure of Levonorgestrel

Levonorgestrel (LNG) has a strong antiestrogenic effect that makes cervical mucus impenetrable to spermatozoa, thus preventing fertilization [1,2]. LNG has also been the progestogen of choice for inclusion in drug delivery systems such as implants, intrauterine devices, and intravaginal rings.

Several analytical methods were described for determination of LNG in biological media or in pharmaceutical dosage forms including difference circular dichroism spectroscopy [3], membrane inlet mass spectrometry and desorption chemical ionization [4], first-derivative spectrophotometry [5], spectrophotometry with multivariate calibration technique [6], liquid chromatography-electrospray-mass spectrometry [7–9], solid-phase extraction-liquid chromatography-diode array-mass spectrometry [10–12], solid-phase gas chromatography-mass spectrometry [13], capillary liquid chromatography and capillary elctrochromatography [14], micellar electrokinetic capillary chromatography [15], and high-performance liquid chromatography with UV-detection [16]. In addition, several methods

* Corresponding author. Tel.: +20-10-6632694; fax: +20-40-3350804.
E-mail address: mmghoneim@usa.net (M.M. Ghoneim).

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were reported for assay of the similar steroidal hormone gestodene including high-performance liquid chromatography with UV-detection [16], miceller electrokinetic capillary chromatography [17,18], partial least-squares and principal component regression multivariate calibration [19], colorimetry, densitometry and derivative spectrophotometry [20], and adsorptive voltammetry at a hanging mercury drop electrode (HMDE) [21]. However, no attempt was made to date to assay levonorgestrel itself using any of the electroanalytical techniques. Most of the reported methods required sample pretreatment and time-consuming solid-phase extraction steps prior to the drug analysis, expensive reagents and equipment, which mostly are not economically feasible for routine use in pharmaceutical analysis and pharmacokinetic studies.

The formulations of such a synthetic steroid with an extremely potent porogestational steroid in tablets of low dosage (e.g., 30–750 μg per table) presents a challenging analytical problem. A sensitive, accurate, and rapid procedure is desired for determination of LNG and testing content uniformity of its dosage form. The LNG structure has a characteristic 3-keto-delta-4-group in the A-ring, which is very promising electroactive center that can be used for the electroanalytical trace determination of this drug in formulations and human serum. In this paper, a direct square-wave adsorptive cathodic stripping voltammetric procedure is described for the trace quantitation of levonorgestrel in bulk form, tablets, and human serum without the necessity for sample pretreatment or time-consuming extraction steps prior to analysis of the drug.

2. Experimental

2.1. Apparatus and software

The cyclic and square-wave voltammetric measurements were carried out using a PAR-Potentiostat/Galvanostat models 263 A or 394 interfaced with a personal computer loaded with the software package 270/250 (PAR). The electrode assembly 303 A (PAR), incorporated with a micro-electrolysis cell consisting of a hanging mercury drop electrode as a working electrode, an $Ag/AgCl/KCl_s$ as a reference electrode and a platinum wire as a counter electrode was used. Stirring of the solution in the electrochemical cell was performed using a magnetic stirrer (305-PAR) and a stirring bar to provide the convective transport during the preconcentration step. The whole measurements were automated and controlled through the programming capacity of the apparatus.

2.2. Reagents

Levonorgestrel was kindly supplied from Schering AG (Berlin, Germany) and was used without further purification. A standard stock solution of LNG (1×10^{-3} M) was prepared by direct dissolution in methanol. The working standard solutions of various concentrations (10^{-6} to 10^{-4} M LNG) were prepared daily by appropriate dilution of the stock solution with methanol. These solutions were protected from light during preparations and measurements. A Mettler balance (Toledo-AB104-Switzerland) was used for weighing the solid materials.

Britton–Robinson (B–R) buffer (pH 2–11) was prepared [22] and used as a supporting electrolyte. An Orion SA 720 pH-Meter, with combined glass and calomel (sat. KCl) electrodes, was used for the pH measurements. The deionized water was supplied from a Purite-Still Plus deionizer connected to a Hamilton-AquaMatic double-distillation water system (UK). All the chemicals (Merck) were of analytical-reagent grade and were used without further purification.

2.3. Procedures

2.3.1. Assay of pure levonorgestrel

A known volume of bulk LNG solution was pipetted into a 10-ml calibrated flask and then completed to the volume with B–R buffer of a selected pH. The percentage of methanol in the final solution was 1%. The solution was introduced into a dark micro-electrolysis cell and then deoxygenated with pure nitrogen for 10 min in the first cycle and for 30 s in each successive cycle; while the nitrogen was then kept over the solution during measurements. The preconcentration of LNG onto the hanging mercury drop electrode surface was performed at −0.8 V (versus $Ag/AgCl/KCl_s$) for a selected duration while stirring the solution at 400 rpm with a magnetic stirrer. After an equilibrium time of 5 s allowed for the solution to become quiescent, the voltammograms were recorded by scanning the potential toward the negative direction using the square-wave waveform under the operational parameters of: pulse-amplitude = 30 mV; frequency = 120 Hz; and scan increment = 10 mV.

2.3.2. Assay of levonorgestrel in tablets

Twenty tablets of Microlut® (Schering AG, Germany), containing 30 μg LNG per tablet, were crushed in a glass mortar to a homogeneous powder. A portion of this powder corresponding to a solution of 1×10^{-5} M LNG was quantitatively weighed and transferred into a 25-ml calibrated flask containing about 15 ml methanol, then sonicated for 10 min, and then completed to the volume with methanol. The content of the flask was then sonicated again for about 10 min to effect dissolution of the drug. Since there were some non-dissolved excipients, the solution was filtered through a 0.45-μm millipore filter. A portion of the clear solution was diluted with the selected supporting electrolyte to the desired concentration. Tablet solutions were analyzed using the same procedure as used in analysis of pure LNG.

2.3.3. Assay of levonorgestrel spiked in human serum

Serum samples were collected from two healthy female volunteers and, then stored frozen until assay. After gentle thawing, aliquots of serum were introduced into seven

centrifugation tubes, then successively spiked with different concentrations of levonorgestrel and then 1.0 ml of methanol (as a precipitating agent of proteins) was added and mixed well. The tubes were vortexed for 3 min at 1500 rpm and then centrifuged for 3 min at 14 000 rpm for separation of the precipitated proteins. The clear supernatant layer was then filtered through a 0.45-μm millipore filter. A 0.1 ml of the supernatant liquor was transferred carefully into the dark electrolysis cell containing 9.9 ml Britton–Robinson buffer of pH 3 as a supporting electrolyte. Following preconcentration of LNG for 90–150 s, the square-wave voltammograms were recorded as in the pure drug.

2.3.4. Pharmacokinetic study

The study was performed on two healthy female volunteers (aged 25–27 years) at Ramadan Specialized Hospital, Tanta City, Egypt. The two volunteers were of regular menstrual cycles and had negative tests for pregnancy. Volunteers refrained from taking any medication including hormonal therapy, from 3 weeks before the study commenced until study completion. Both volunteers fasted overnight for 8 h before dosing. The two volunteers gave their written informed consent prior to participating in the study (no permission is required from any ethical committee). Following an oral administration of a 30-μg LNG single dose, 5 ml of venous blood samples were aseptically aspirated from each volunteer at different time periods over 36 h and collected in appropriately labeled lithium-heparin tubes. The blood samples were centrifuged immediately at 2000 rpm for 10 min, and the plasma fractions were rapidly separated and stored in coded polypropylene tubes at −20 °C until assayed. Each plasma sample was assayed in duplicate using the proposed square-wave adsorptive cathodic stripping voltammetric procedure (t_{acc} = 150 s at −0.8 V), and the mean of the two values provides the plasma concentrations at the time period of collection of the blood sample.

To study the reproducibility, accuracy and precision of the results of the proposed procedure for analysis of LNG in bulk substance, tablets and human serum or plasma samples, recovery experiments were carried out by means of both the calibration curve and standard addition methods.

3. Results and discussion

3.1. Cyclic voltammetry

The cyclic voltammograms of 1×10^{-4} M LNG in B–R buffers of pH 2–11 at the HMDE showed a single two-electron irreversible cathodic peak over the whole pH range. This peak may be attributed to the reduction of the 3-keto-delta-4-group in the A-ring of the LNG molecule. No oxidation peak was observed in the anodic direction, indicating the irreversible nature of the reduction process of LNG. The peak potential shifted linearly to more negative values with the increase of scan rate, which also confirmed

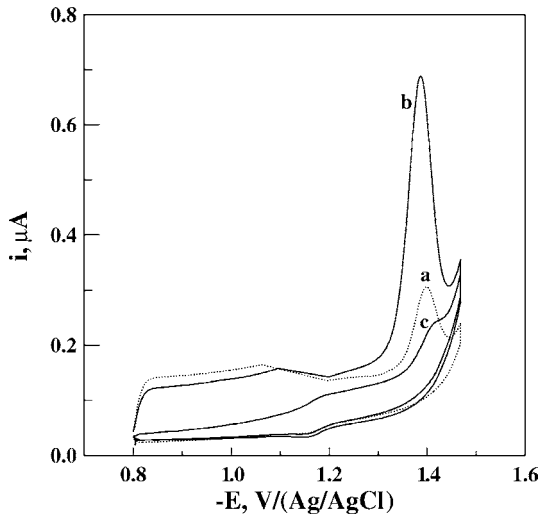

Fig. 1. Cyclic voltammograms of 1×10^{-6} M LNG: (a) without preconcentration; t_{acc} = 0 s, (b) following preconcentration for 30 s at E_{acc} = −0.8 V, and (c) repetitive cycle of (b) at the same mercury drop; scan rate = 200 mV/s.

the irreversible nature of the electrode reaction. Moreover, the peak current increased with the increase of scan rate, which is the behavior expected for the mass transport controlled by adsorption [23]. A linear displacement of the peak potential to more negative values with the pH of the medium was obtained following the relation $E_p = 0.072\,\mathrm{pH} + 1.14$. The slope value of 72 mV/pH indicates the involvement of protons in the electrode reaction, and that the proton-transfer reaction precedes the electrode process proper [24].

The interfacial adsorptive character of LNG at the hanging mercury electrode surface was identified by the repetitive cyclic voltammograms recorded before and following preconcentration of LNG for 30 s at −0.8 V (Fig. 1). The preconcentration of LNG onto the HMDE surface generated a substantial enhancement of the cathodic peak (Fig. 1, curve b) compared to that of the subsequent scan at the same mercury drop (Fig. 1, curve c), thus indicating a rapid desorption of drug species out of the mercury electrode surface.

Adsorptive stripping cycles carried out in B–R buffers of pH 2–3 for increased values of scan rate (ν) gave rise to a reduction peak current with intensity that showed a linear increase with scan rate (25–500 mV s^{-1}). The plot of log i_p versus. log ν was a straight line following the relation: $\log i_p(\mu A) = 0.97 \log \nu - 2.0$ ($r = 0.998$); its slope (0.97) is very close to 1.0, which is the expected value for an ideal reaction of surface species [25]. The dependence of the peak potential on the decimal logarithm of the scan rate was also linear according to the equation: $E_p(\mathrm{mV}) = -12.7 - 45.3 \log \nu$ (mV s^{-1}). From its slope, a value of $\alpha n_a = 1.3$ was obtained.

3.2. The electrode surface coverage

The electrode surface coverage (Γ_0, mol cm^{-2}) with the drug species can be determined from the amount of

charge (Q) consumed by the surface process as calculated by the integration of the area under the peak of the cyclic voltammogram corrected to residual current [26]. The surface coverage Γ_0 (the amount of reactant adsorbed onto the mercury electrode surface, mol cm^{-2}) can be calculated using the equation $\Gamma_0 = Q/nFA$ where n is the number of electrons consumed in the reduction process ($n = 2$), F is the Faraday's constant (96487 C) and A is the electrode surface area (0.026 cm^2). On dividing the number of coulombs transferred, 1.01×10^{-6} C, by the conversion factor nFA (5017.3 C), a monolayer surface coverage of 2.013×10^{-10} mol cm^{-2} was obtained in B–R buffer of pH 3. Thus, each adsorbed LNG molecule therefore occupies an area of 0.825 nm^2.

Furthermore, the total charge (Q_{total}) versus the square root of time ($t^{1/2}$) plots of the blank (B–R buffer of pH 3) and 5×10^{-5} M LNG following preconcentration of the drug onto the HMDE for 30 s were recorded using chronocoulometric measurements. The amount of reactant adsorbed onto the mercury electrode surface Γ_0 (mol cm^{-2}), was estimated on applying the following relation [27]:

$$Q_{total} = \int_0^t i\mathrm{d}t = \frac{2nFACD^{1/2}t^{1/2}}{\pi^{1/2}} + nFA\Gamma_0 + Q_{dl}$$

The measured total charge (Q_{total}) represents contribution from three possible sinks: (i) electrolysis of electroactive species in solution at a rate that is controlled by diffusion to the electrode ($Q_{diff} = 2nFACD^{1/2}t^{1/2}/\pi^{1/2}$), (ii) electrolysis of electroactive species that is adsorbed onto the electrode surface ($Q_{ads} = nFA\Gamma_0$), and (iii) charging of the electrode–electrolyte (blank) double layer capacitance to the new potential (Q_{dl}), i.e.,

$$Q_{total} = Q_{diff} + Q_{ads} + Q_{dl}$$

The charge (Q_{total}) versus $t^{1/2}$ plot has an intercept equal to ($nFA\Gamma_0 + Q_{dl}$). Since Q_{dl} can be measured in a separate experiment for the supporting electrolyte alone (blank), the contribution of the adsorbed species can be determined and Γ_0 can be calculated. In the present work, the difference between the intercepts of the charge–$t^{1/2}$ plots for Q_{total} and Q_{dl} was found to equal 1.022 μC, which is the amount of charge required to reduce the adsorbed reactant species (Q_{ads}). So, the surface coverage Γ_0 was found to equal 2.036×10^{-10} mol cm^{-2}. Thus, each adsorbed LNG molecule therefore occupies an area of 0.815 nm^2, which agrees well with that obtained by means of cyclic voltammetric measurements under the same experimental conditions. The diffusion coefficient (D°) of LNG in B–R buffer of pH 3 was also estimated from the slope value of the Q_{total} versus. $t^{1/2}$ plot and was found to equal 1.3×10^{-8} cm^2 s^{-1}.

3.3. Square-wave voltammetry

The square-wave voltammograms of 5×10^{-7} M LNG in B–R buffers (pH 2–11) were recorded without preconcentration and following preconcentration for 30 s. Following preconcentration onto the HMDE a well-defined single peak was observed over the pH range 2–7. The i_p versus. pH plots (Fig. 2) indicated that the response preceded by preconcentration increased extensively, and a much more developed peak current intensity was observed at pH 3. So, a B–R buffer of pH 3 was chosen as a supporting electrolyte throughout the present analytical study. The dependence of the peak current of 1×10^{-7} M LNG on the accumulation potential was also evaluated over the range −0.3 to −1.2 V in B–R of pH 3 following preconcentration for 90 s. At a potential of −0.8 V, a much more developed peak current was achieved.

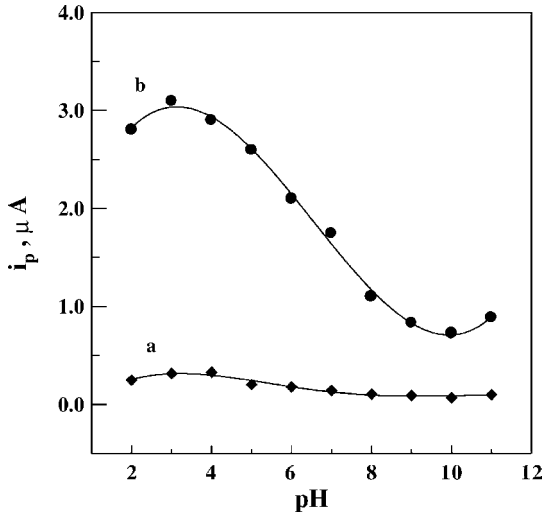

Fig. 2. Dependence of square-wave peak current upon pH, for 5×10^{-7} M LNG. (a) $t_{acc} = 0$ s, (b) $t_{acc} = 30$ s; $E_{acc} = -0.8$ V, frequency $f = 120$ Hz, scan increment $\Delta E = 10$ mV, pulse amplitude $a = 30$ mV, stirring rate = 400 rpm, and rest period = 5 s.

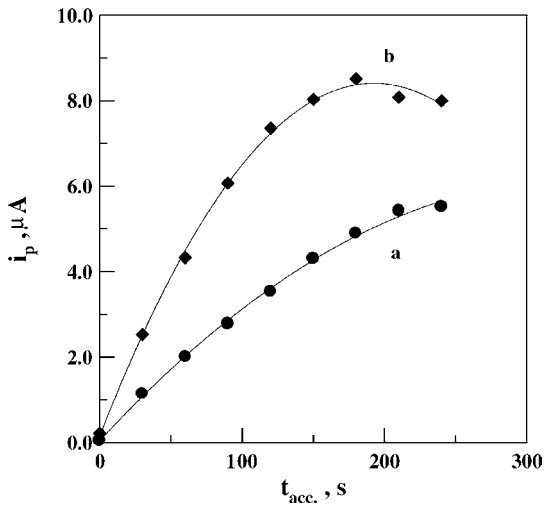

Fig. 3. Effect of accumulation time (t_{acc}) on SWAdCS voltammetric peak current of: (a) 5×10^{-8} M and (b) 1×10^{-7} M LNG in B–R buffer of pH 3, $E_{acc} = -0.8$ V. Other operational parameters are as those indicated in Fig. 2.

Table 1
Characteristics of the calibration plots of bulk levonorgestrel under the operational conditions of the proposed SWAdCS voltammetric procedure following different preconcentration time periods

t_{acc} (s)	Regression equation i_p (μA) = bC (nM) + a	Linearity range	Correlation coefficient (r)	LOD (M)	LOQ (M)
10	$i_p = 0.018\ C - 0.17$	1×10^{-8} to 2×10^{-7}	0.998	2.1×10^{-9}	7.0×10^{-9}
30	$i_p = 0.032\ C - 0.23$	9×10^{-9} to 2×10^{-7}	0.997	1.3×10^{-9}	4.3×10^{-9}
90	$i_p = 0.067\ C - 0.26$	5×10^{-9} to 1×10^{-7}	0.996	6.7×10^{-10}	2.2×10^{-9}
150	$i_p = 0.093\ C - 0.29$	1×10^{-9} to 1×10^{-7}	0.997	4.8×10^{-10}	1.6×10^{-9}

The effect of accumulation time on the peak current of 5×10^{-8} and 1×10^{-7} M LNG was studied at −0.8 V. The peak current increased linearly with the increase of accumulation time over the period 10–150 s (Fig. 3). Thus, the accumulation time of choice will be dictated by the sensitivity needed to assay LNG in different samples. The plot of peak current (i_p) versus the square root of time ($t^{1\backslash 2}$) gave a straight line with slope of 0.37; this behavior is expected for mass transport controlled by adsorption [28].

The stripping peak current of 1×10^{-7} M LNG in B–R buffer of pH 3 (t_{acc} = 90 s and E_{acc} = −0.8 V) was optimized by changing the pulse-amplitude a (10–100 mV), scan increment ΔE (2–10 mV), and frequency f (10–120 Hz). The dependence of the peak current (i_p) on the square wave frequency (f) was linear. The peak current increased also with the increase of pulse-amplitude. However, a pulse-amplitude of 30 mV was applied throughout the present study, at which a much sharper peak with smaller width was observed. Also, the stripping peak current increased linearly as the scan increment (ΔE) increased. A scan increment of 10 mV was chosen in the present study. Thus, the optimal operational conditions of the developed square-wave adsorptive cathodic stripping (SWAdCS) voltammetric procedure were: B–R buffer of pH 3, accumulation potential = −0.8 V, accumulation time = 10 − 150 s, frequency = 120 Hz, scan increment = 10 mV, and pulse-amplitude = 30 mV using an HMDE of area = 0.026 cm^2.

3.4. Calibration curves and validation of the procedure

The calibration curves for LNG determination following preconcentration for different time periods were established applying the developed procedure. The slope values of the calibration curves over different concentration ranges of LNG can be different because the peak heights are affected by the kinetics of the reaction, which may be changed with the augmentation of analyte concentration and the preconcentration time. The characteristics of the calibration curves are reported in Table 1. Limits of detection (LOD) and quantitation (LOQ) of bulk LNG following different preconcentration time periods were estimated (Table 1) using the following equations [29]:

$$\text{LOD} = \frac{3s}{m} \quad (1)$$

$$\text{LOQ} = \frac{10s}{m} \quad (2)$$

where s is the standard deviation of the intercept and m is the slope of the calibration curve.

The reproducibility, precision, and accuracy of measurements by means of the proposed procedure were identified by performing seven successive determinations of three different concentrations of bulk LNG over one day (intraday assay) and over a week (inter-day assay), and the results obtained are reported in Table 2.

Evaluating the influence of small variation in some of the most important procedural conditions, including pH (−2.5 to 3.5) and preconcentration potential (7–9 V), examined the robustness [30] of the procedure. The results showed that none of these variables significantly affect the recovery of LNG and provide an indication of the reliability of the proposed procedure for its assay. Thus, the proposed procedure could be considered robust. Moreover, the ruggedness [30] of the measurements was examined by applying the optimized procedure to assay bulk LNG using two electrochemical analyzers models 263A and 394-PAR under the

Table 2
Analytical precision and accuracy of assay of bulk levonorgestrel by the proposed voltammetric procedure, following preconcentration for 90 s

Concentration (Taken ng ml^{-1})	Intraday[a]				Interday[b]			
	Concentration (found ng ml^{-1})	R (%)	Bias (%)	R.S.D. (%)	Concentration (found ng ml^{-1})	R (%)	Bias (%)	R.S.D. (%)
3	2.97	99.0	−1.0	1.7	2.92	97.3	−2.7	1.0
9	9.11	101.2	1.2	1.1	9.04	100.4	0.4	0.9
21	21.09	100.4	0.4	0.8	21.02	100.1	0.1	0.5

For intraday F-value = 2.97 and t-value = 1.36. For interday F-value = 1.48 and t-value = 2.05
[a] Average of seven measurements over a day.
[b] Average of seven measurements over a week.

Table 3
Assay of Microlut® tablets by the proposed SWAdCS voltammetric procedure following preconcentration for 30 s; using the standard addition method

Concentration (taken ng ml^{-1})	Concentration (found[a] ng ml^{-1})	R (%)	R.S.D. (%)	Bias (%)
6.25	6.42	102.78	1.32	2.72
12.50	12.67	101.36	0.96	1.36
18.75	18.97	101.17	1.29	1.17

F-value = 3.35 and t-value = 1.27.
[a] Average of five measurements.

same optimized experimental conditions at different elapsed time. The recoveries obtained because of lab-to-lab and even day-to-day variations were found reproducible, since there was no significant difference in the recovery and standard deviation results.

3.5. Application to tablets

In order to identify the extent of interference from excipients present in Microlut® tablets, recovery tests were evaluated after addition of known amounts of pure LNG to various preanalyzed solutions of LNG formulation. The results indicated that the proposed procedure can be applied to assay of LNG in tablets with a great success without interference from excipients, following its preconcentration onto the HMDE for 10–30 s. At longer preconcentration time periods, interference from excipients was observed. The results of analysis of tablets (Table 3) are favorably compared to those achieved by a reported method [5]. The mean recoveries (R%) based on the average of seven replicate measurements on the same batch of sample following preconcentration for 10 or 30 s were calculated with regard to the claimed value of the manufacturer. The results showed that the calculated F- and t-values [24] did not exceed the theoretical values (95% confidence limits for five degree of freedom), from which it can be concluded that precision of the proposed procedure did not differ significantly from the reported method [5]. However, the proposed stripping procedure is less costly, faster, and no sample pretreatment or time-consuming extraction or evaporation steps are required prior to assay of the drug.

3.6. Application to human serum

The proposed SWAdCS voltammetric procedure was also successfully applied for the quantitation of LNG spiked in human serum (representative voltammograms are shown in Fig. 4) without the necessity for sample pretreatment or time-consuming extraction or evaporation steps prior to the analysis, other than the centrifugal separation of proteins precipitated by methanol from the serum (or plasma) samples. The characteristics of calibration plots for assay of the spiked serum samples at different accumulation duration are reported in Table 4. The estimated lower LOD and LOQ of LNG in spiked human serum were found to be 7×10^{-10} and 2.3×10^{-9} M, or 5.7×10^{-9} and 1.9×10^{-9} M following preconcentration onto the hanging mercury drop electrode for 90 or 150 s, respectively. The intraday and interday precision was evaluated by seven replicate measurements of serum samples spiked with LNG (Table 5).

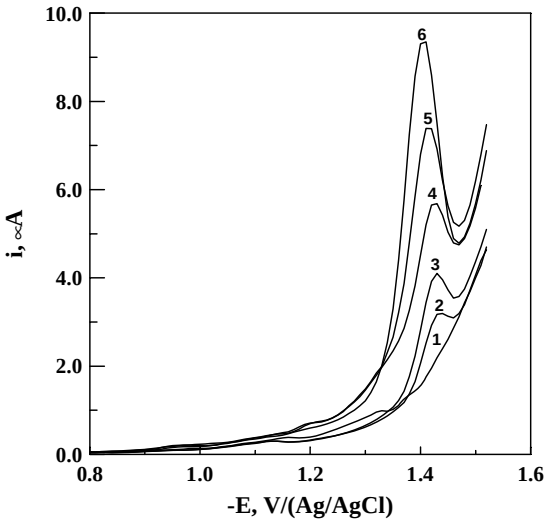

Fig. 4. Representative SWAdCS voltammograms for different concentrations of LNG spiked in human serum: (1) background; (2) 2×10^{-9}; (3) 8×10^{-9}; (4) 2×10^{-8}; (5) 4×10^{-8}; and (6) 6×10^{-8} M LNG following preconcentration for 150 s Operational parameters are as those indicated in Fig. 2.

3.7. Pharmacokinetic study

The developed procedure was applied to determination of the drug in real human plasma samples following preconcentration onto the HMDE for 150 s. The time course of LNG concentrations was studied in plasma samples, collected from two female volunteers at specified intervals, following an oral administration of a 30-μg LNG single dose (Microlut® tablet). The obtained plasma concentration–time

Table 4
Characteristics of the calibration plots of levonorgestrel spiked in human serum under the operational conditions of the proposed SWAdCS voltammetric procedure following different preconcentration time periods

t_{acc} (s)	Regression equation $i_p(\mu A) = bC$ (nM) + a	r	Linearity range	LOD (M)	LOQ (M)
30	$i_p = 0.0297\ C - 0.51$	0.996	5×10^{-8} to 2×10^{-7}	1.5×10^{-9}	5.0×10^{-9}
90	$i_p = 0.0639\ C - 0.63$	0.998	1×10^{-8} to 1×10^{-7}	7.0×10^{-10}	2.3×10^{-9}
150	$i_p = 0.0795\ C - 0.32$	0.996	1×10^{-9} to 9×10^{-8}	5.7×10^{-10}	1.9×10^{-9}

Table 5
Recovery, precision and accuracy of assay of levonorgestrel spiked in human serum by the proposed procedure following preconcentration for 90 s

Conccentration (taken ng ml^{-1})	R (%)	Intraday[a]		Interday[b]	
		Bias (%)	R.S.D. (%)	Bias (%)	R.S.D. (%)
6	96.18	−3.12	1.14	−2.88	1.33
12	97.56	2.32	0.94	1.52	1.69
25	98.64	0.64	0.72	1.56	0.95

[a] Average of seven measurements over a day.
[b] Average of seven measurements over a week.

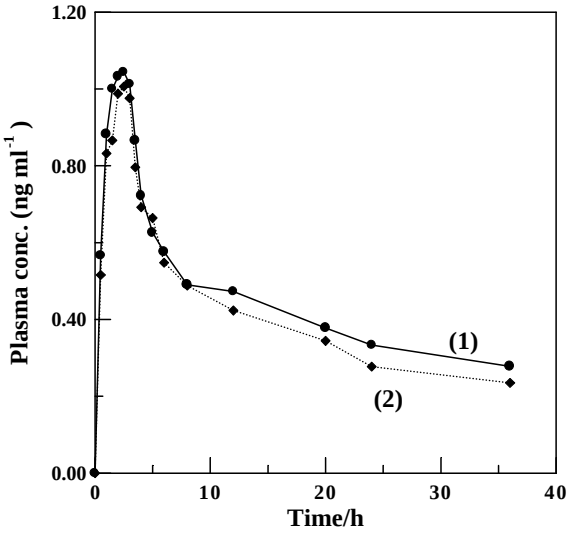

Fig. 5. Plasma concentration–time profile for two healthy female volunteers (1 and 2) after an oral administration of a 30-μg LNG single dose. Analysis of the drug was carried out by the proposed SWAdCS voltammetric procedure (B–R buffer of pH 3, preconcentration for 150 at −0.8 V). Other operational parameters are as those indicated in Fig. 2.

profiles (Fig. 5) of the two volunteers were used for estimation of the pharmacokinetic parameters of LNG drug which are: the maximum plasma concentration (C_{max}), the time (t_{max}) to reach C_{max}, and the area under the plasma concentration–time profile ($AUC_{0\text{-}t}$) calculated from time 0 until the last time point t. The estimated mean pharmacokinetic parameters were found to equal: $C_{max} = 1.05\,ng\,ml^{-1}$, $t_{max} = 2.4\,h$, and $AUC_{0\text{-}t} = 16.5\,ng\,h\,ml^{-1}$. The obtained results were favorably compared to those reported in literature for equivalent LNG single dose [31,32]. These results supplied an evidence for the reliability of the proposed procedure for assay of levonorgestrel drug in real blood samples.

4. Conclusion

The high sensitivity of adsorptive stripping voltammetry technique makes it possible to work with very diluted samples with a corresponding decrease in possible interference in the analysis. A sensitive and practical square-wave adsorptive stripping voltammetric procedure for determination of levonorgestrel at ng ml^{-1} level is described. The procedure is successfully applied to assay of levonorgestrel in tablets and human blood. Furthermore, the analysis time was short and the procedure had adequate precision and accuracy, and consequently is strongly recommended for analysis of LNG in clinical and quality control laboratories.

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