Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
34 (2004) 75–86
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Voltammetric behavior and quantification of the sedative-hypnotic drug chlordiazepoxide in bulk form, pharmaceutical formulation and human serum at a mercury electrode

G.B. El-Hefnawey*, I.S. El-Hallag, E.M. Ghoneim, M.M. Ghoneim

Chemistry Department, Faculty of Science, Tanta University, El-Bahr Street, 31572 Tanta, Egypt

Received 3 June 2003; received in revised form 1 August 2003; accepted 2 August 2003

Abstract

Chlordiazepoxide is a sedative-hypnotic drug widely employed as a transquilizer and anti-depressant. Its electrochemical behavior in Britton–Robinson (B–R) buffers of pH 2–11 at a mercury electrode has been investigated using dc-polarography, cyclic voltammetry and controlled-potential coulometry. Polarograms of the drug in B–R buffers of pH 2–10 exhibited three 2-electron waves, while at pH $>$ 10, only a single 4-electron wave was observed. The first, second, and third waves in buffers of pH $\leq$ 10 may be attributed to the reduction of N-oxide, C=N and C–N centers, respectively. The single wave (pH $>$ 10) may be due to the reduction of both the N-oxide and C=N centers in a one step. The shift of the $E_{1/2,}$ values to more negative potentials upon the increase of pH indicated the involvement of protons in the electrode reaction and that the proton-transfer reaction precedes the electrode process proper. The estimated data indicated that, one proton and two electrons are participated in the rate-determining step of each of the reduced centers. The general sequence of chlordiazepoxide reduction processes via each of its reactant centers may be expressed as: H^+, e, e, $H^+_{(fast)}$.

Based on the interfacial adsorptive character of the drug onto the mercury electrode, a validated direct square-wave adsorptive cathodic stripping (SWAdCS) voltammetric procedure has been described for the trace determination of the drug in bulk form, tablets and human serum. The procedure did not require sample pretreatment or time-consuming extraction or evaporation steps prior to the assay of the drug. The optimized operational conditions of the proposed procedure have been found to be: accumulation potential $E_{acc.} = -0.9$ V, accumulation time $t_{acc.} = 30$ s, pulse-amplitude $= 50$ mV, scan increment $= 10$ mV and frequency $= 120$ Hz. The proposed procedure is much more simple, fast, sensitive, costly low and achieved much more lower limits of detection (LOD) (4.4×10^{-10} M and 6.6×10^{-10} M) and limits of quantitation (LOQ) (1.5×10^{-9} M and 2.2×10^{-9} M), respectively in pharmaceutical formulation and spiked human serum, compared to previously reported methods.

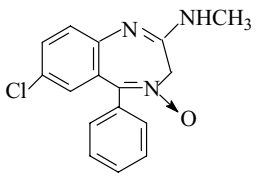
Keywords: Chlordiazepoxide drug; Electrochemical behavior; dc-Polarography; Cyclic voltammetry; Controlled-potential coulometry; Quantification; Square-wave; Adsorptive stripping voltammetry; Librax tablets; Human serum

1. Introduction

Chlordiazepoxide (7-chloro-*N*-methyl-5-phenyl-3H-1,4-benzodiazepine-2-amina-4-oxide) (Scheme 1)

* Corresponding author. Tel.: +20-40-3350804; fax: +20-40-3350804.
E-mail address: gadbelhefnawy@hotmail.com (G.B. El-Hefnawey).

0731-7085/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.japna.2003.08.008



Scheme 1.

is used as anxiolytics, sedatives, hypnotics, anticonvulsants, and/or skeletal muscle relaxants. It shares the actions of other benzodiazepines and is used for the management of anxiety disorders or for short-term relief of symptoms of anxiety, preoperatively to relieve anxiety and tension, and for the management of agitation associated with acute alcohol withdrawal.

The drug may inhibit monosynaptic and polysynaptic reflexes by acting as inhibitory neuronal transmitters or by blocking excitatory synaptic transmission. The drug may also directly depress motor nerve and muscle function [1,2]. Clidinium bromide (3-[(hydroxy-diphenylacetyl)-oxy]-1-methyl-1-azoniabicylo-[2.2.2]octane bromide is effective for anxiety-related conditions including spastic colon [3]. Librax is a pharmaceutical formulation which contains both drugs.

For clinical investigations such as pharmacokinetic studies, developments of sensitive and selective analytical methods to the determination of drugs in biological fluids are highly required. Several analytical methods were described for the determination of chlordiazepoxide in pharmaceutical formulations and plasma samples. These include spectrophotometry [4–8], spectrofluorimetry [9,10] reversed-phase high-performance liquid chromatography [11–17], high-performance thin layer chromatography [18], thin layer chromatography [19], liquid chromatography [20,21] and reduction by zinc or cadmium in a continuous system followed by atomic-absorption spectrometric detection [22].

Since all benzodiazepines possess an azomethine functional group in position four of the benzodiazepine ring that can be easily reduced at a mercury electrode, electrochemical techniques were used quite efficiently for solution studies as well as for quantitative determination of many of these compounds [23]. Because the presence of phenyl rings, benzodiazepines have surface activity and are readily adsorbed onto mercury surface, it is, therefore, not surprising that among other methods that were used to determination of bezodiazepines [4–22] the electrochemical techniques using a mercury electrode seems to be efficient not only for solution studies but also for quantitative determination [23,24].

The polarographic behavior of chlordiazepoxide and its reduction mechanism at a mercury electrode were studied in different media [25–30]. Chlordiazepoxide was determined in different samples using several electrochemical techniques, e.g. dc-polarography [30–34], differential-pulse polarography [35,36], differential-pulse stripping voltammetry [37] and square-wave polarography and voltammetry [38].

Most of the reported methods [4–22] to determination of chlordiazepoxide required pretreatment and time-consuming extraction or evaporation steps prior to the analysis, which considered as disadvantages of the trace analysis methods. Furthermore, the reported polarographic procedures [30–34] for determination of the drug in pharmaceutical formulation and toxicological samples are not sufficiently sensitive for convenient application to human biological fluids. Moreover, some of the polarographic and voltammetric procedures [36,37] also required pre-separation of the drug prior to the analysis.

Although, the polarographic behavior of chlordiazepoxide was investigated in different media [25–30], however the reported results have some discrepancies concerning both the reducible centers and the number of protons transferred in the electrode process.

Stripping analysis is an extremely sensitive technique that utilizes a bulk electrolyte step to preconcentrate the analyte from the sample solution into or onto the working electrode. Most stripping measurements involve peconcentration into or onto mercury electrode. In this case, the preconcentration step can be viewed as an effective electrochemical extraction in which the analyte is preconcentrated into or onto the mercury phase to a much higher level than it exists in solution. The preconcentration step is followed by an electrochemical measurement of the concentrated analyte. This combination of a preconcentration step with advanced measurement procedures generates the extremely favorable signal-to-background ratio that characterizes stripping analysis [39]. Adsorptive stripping analysis greatly enhances the scope of stripping measurements toward various organic compounds. The high sensitivity and low cost of stripping analysis

have led to its application in a large number of analytical problems [39]. This technique did not require sample pretreatment or time-consuming extraction or evaporation steps prior to the drug assays.

The square-wave adsorptive stripping voltammetry has been the analytical tool used in the present study, since no attempt was made yet to assay the chordiazepoxide drug using this technique. Square-wave voltammetry is a large-amplitude differential technique in which a waveform composed of a symmetrical square-wave, is applied to the working electrode [39,40]. The current is sampled twice during each square-wave cycle, once at the end of the forward pulse and once at the end of the reverse pulse. The difference between the two measurements is plotted versus the base staircase potential. The resulting peak current is proportional to the concentration of the analyte. Excellent sensitivity accrues from the fact that the net current is larger than either the forward and reverse components. Coupled with the effective discrimination against the charging current, very low detection limits can be attained. Comparison of square-wave and differential-pulse voltammetry for reversible and irreversible cases indicated that the square-wave current are 4 and 3.3 times higher, respectively, than the analogous differential-pulse response [40,41]. The major advantage of square-wave voltammetry is its great speed. The effective scan rate is given by square-wave frequency f (Hz) and the step height ΔE_s.

This work throws a more deep light upon the electrochemical behavior and elucidation of the electrode reaction pathway of chlordiazepoxide at the mercury electrode. A validated square-wave adsorptive stripping voltammetric procedure is also described for the trace determination of the drug in bulk form, pharmaceutical formulation and human serum, without the need for sample pretreatment or time-consuming extraction or evaporation steps prior to the drug analysis.

2. Experimental

2.1. Chemicals and solvents

Bulk chlordiazepoxide was supplied from Hoffmann-La Roche. Its Librax tablets (Hoffmann-La Roche) labeled to contain 5 mg chlordiazepoxide and 2.5 mg clidinium bromide. Analytical grade, boric acid, phosphoric acid, acetic acid and sodium hydroxide were used for preparation of the Britton–Robinson (B–R) buffers of pH 1.9–11 [42]. The pH of the buffers was checked using a pH-meter (Knick) of sensitivity ±0.02 pH units. Deionized water used for preparation of solutions was obtained from a Purite Still Plus HP deionizer connected to a Hamelton AquaMatic bidistillation water system. Ethanol (Merck) was used for preparation of stock solutions of the drug.

2.2. Reagents and solutions

A stock solution of 1×10^{-3} M bulk chlordiazepoxide was prepared by dissolving an accurate quantity of the drug in a specific volume of ethanol, kept in a dark glass bottle and then stored at 4 °C. More diluted solutions (10^{-4}–10^{-6} M) of bulk chlordiazepoxide were prepared by accurate dilution with ethanol. The solutions were stable and their concentrations did not change with time for at least 2 weeks.

Ten Librax tablets were weighed and the average mass per tablet was determined. A quantity of finely grounded material equivalent to 30 mg of chlordiazepoxide was accurately weighed, and transferred into a 100 ml calibrated flask contained 70 ml ethanol. The contents of the flask were shaken for 15 min and then completed to the volume with ethanol. The solution was next filtered through a 0.45 μm Millipore filter (Gelman, Germany). Aliquots of this solution were diluted with ethanol to produce 10^{-4}–10^{-6} M chlordiazepoxide solutions.

Serum samples were obtained from healthy volunteers and stored frozen until the assay. An aliquot of the standard stock chlordiazepoxide solution was fortified with the human serum sample. A 200 μl aliquot of the spiked human serum sample was diluted to a 1.0 volume with ethanol in a 2 ml volume centrifuge tube. The precipitated proteins were separated by centrifugation for 5 min at 14 000 rpm. An aliquot of the clear supernatant solution was diluted to 10 ml with B–R buffer, then transferred into a dark micro-electrolysis cell.

2.3. Instrumentation

A pen-recording polarograph (model 4001 Sargent–Welch) and the polarographic cell described by Meites

[43] were used for the present polarographic measurements. The characteristics of the dropping mercury electrode were: $m = 1.35\,\mathrm{mg\,s^{-1}}$ and $t = 3.4\,\mathrm{s}$ (at 60 cm mercury height). A saturated calomel electrode (SCE) was used as a reference electrode.

The voltammetric behavior of the drug was studied using the Electrochemical Trace Analyzers models 394 and 263A (PAR). The 303A-PAR electrode assembly, incorporated with a three electrode system comprising of a hanging mercury drop electrode (HMDE) (area $= 2.6 \times 10^{-2}\,\mathrm{cm^2}$) as a working electrode, a Pt wire as a counter electrode and an $Ag/AgCl/KCl_s$ as a reference electrode, was used for the voltammetric measurements. A magnetic stirrer (305-PAR) with a star-shaped magnet was used to provide the convective transport during the preconcentration step. The whole measurements were automated and controlled through the programming capacity of the apparatus. The data were treated through a PC connected to the Electrochemical Analyzer using the 394 Analytical voltammetry software version-2.01 copyright 1994.

The potentiostat/galvanostat model 173-PAR, incorporated with a digital coulometer model 179-PAR, and a dark coulometric cell comprising of a mercury pool (as a working electrode), a saturated calomel electrode (as a reference electrode) and a platinum gauze immersed in a bridge tube (as a counter electrode), was used for the controlled-potential electrolysis of the drug. The potential selected was adjusted to a value equal to the $E_{1/2}$ of the polarographic wave of the reactant plus 100 mV or at the beginning of its limiting current. The total charge passed during the exhaustive electrolysis was obtained by integrating the current electronically.

Faraday's law related the measured charge Q (Coulombs) transferred to the amount of material electrolyzed as: $N = Q/nF$, where N is the number of moles of substance being electrolyzed, n the number of electrons transferred per reactant molecule, and F the Faraday's constant. The charge Q (Coulombs) was read directly from the digital coulometer. The total number of electrons (n) consumed for the overall reduction reaction of the drug were found to equal 6 or 4-electrons/drug molecule over the pH range 2–10 or higher (pH $>$ 10), respectively. In solutions of pH $\leq$ 10, two electrons were consumed via each of the first, second and third waves, while in solutions of higher pH values, four electrons were consumed via the single wave.

A Mettler balance (Toledo-AB104) was used for weighing the solid materials. An Eppendorf centrifuge 5417C was used for separation of the precipitated protein from the serum samples before the drug assays.

2.4. Procedures

For polarographic and cyclic voltammetric measurements, a known volume of the drug solution was pipetted into a 10 ml volume calibrated flask and then completed to the volume with B–R buffer. The solution was transferred into the electrolysis cell, then deoxygenated with pure nitrogen for 10 min and a nitrogen atmosphere was maintained over the solution while recording the polarograms/voltammograms.

For stripping voltammetry measurements, a known volume of the drug solution was pipetted into a 10 ml volume calibrated flask and then completed to the volume with B–R buffer of pH 8. The solution was introduced into a dark micro-electrolysis cell, then deoxygenated with pure nitrogen for 10 min. The selected accumulation potential was applied at the working electrode for a selected time while the solution was stirred. At the end of the accumulation time period the stirrer was stopped and 10 s was allowed for the solution to become quiescent. Then the voltammograms were recorded by scanning the potential toward the negative direction applying the square-wave waveform. All data were obtained at room temperature.

3. Results and discussion

3.1. dc-Polarographic studies

The dc-polarograms of chlordiazepoxide in B–R buffers of pH values (2–10) exhibited three irreversible reduction waves of almost equal heights. In solution of pH $>$ 10, only a single wave was observed; its height was equal to the sum of both heights of the first and second waves (Fig. 1). The first wave (pH $\leq$ 10) may be attributed to 2-electron reduction process of the N-oxide group, while the second and third waves (pH $\leq$ 10) may be due to the saturation of the C=N and cleavage of C–N centers, respectively, via the

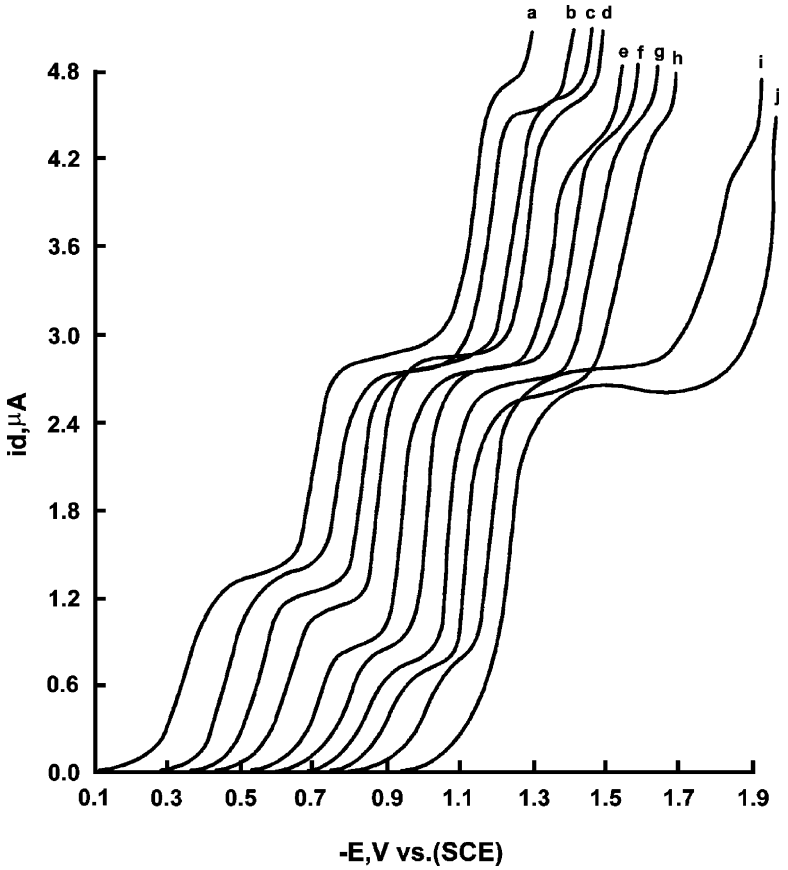


Fig. 1. dc-Polarograms for 2.5×10^{-4} M bulk chlordiazepoxide in B–R buffers of different pH values: (a) 1.9, (b) 2.9, (c) 3.9, (d) 4.9, (e) 5.9, (f) 6.9, (g) 8, (h) 9, (i) 10, and (j) 11.

consumption of two electron for each one. At pH > 7, the $E_{1/2}$ of the second wave was less pH-dependent than that of the first one, therefore both coalesced in a single 4-electron wave at pH > 10 due to the reduction of both N-oxide and C=N centers in one step ($E_{1/2(1)} = E_{1/2(2)}$). While the third wave disappeared in buffers of pH > 10, which may be due to its overlap with that of the medium discharge. The total limiting current was almost pH-independent up to pH 10, then decreased at higher pH values to 2/3 of its value (Fig. 2). This behavior was confirmed by means of the complete electrolysis of the drug at a controlled-potential in B–R buffers (pH 2–11), since 6 or 4-electrons/drug molecule were consumed for the total reduction process of the drug in solutions of pH ≤10 or >10, respectively. The half-wave potentials of the three reduction waves shifted to more negative values upon the increase of pH. This behavior indicated the involvement of protons in the electrode reaction and that the proton-transfer reaction precedes the electrode process proper [44]. On the other hand, the co-formulated clidinium bromide compound was found to be electro-inactive at the mercury electrode under the present conditions of investigation, hence it did not interfere with the electrode reaction of chlordiazepxide.

According to the fundamental equation of the irreversible polarographic waves [43], the plots of E_{de} versus $\log(i/i_d - i)$ for the three waves at different pH values were straight lines of slope values S_1 ($S_1(\text{mV}) = 59/\alpha n_a$) reported in Table 1. The estimated values of αn_a and the transfer coefficient α (Table 1) denoted the irreversible nature of the reduction processes of N-oxide, C=N and C–N centers

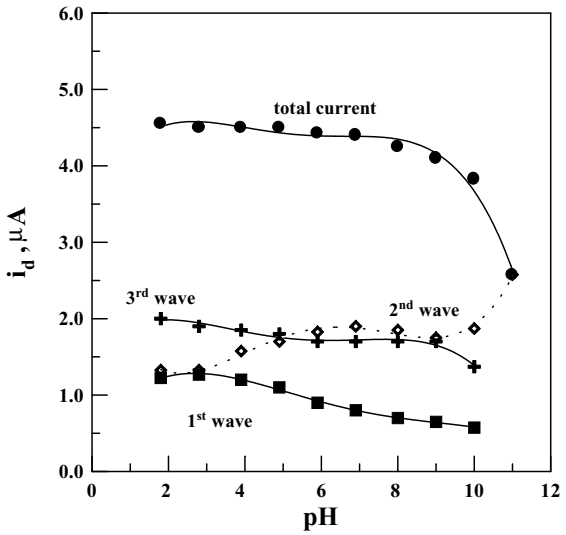


Fig. 2. The polarographic i_d–pH plots for the reduction waves of 2.5×10^{-4} M chlordiazepoxide.

and that the number of electrons n_a transferred in the rate-determining step are equal for the three processes, and found to be 2.

Also the plots of $E_{1/2}$–pH of the reduction waves of chlorodiazepoxide were straight lines of slope values S_2 reported in Table 1. The number of hydrogen ions (Z_{H^+}) participated in the rate-determining step was calculated using the slope values S_1 and S_2 of both the E_{de}–log$(i/i_d - i)$ and $E_{1/2}$–pH plots, respectively, as [43,45]

$$\frac{\partial E_{1/2}}{\partial \mathrm{pH}} = \left(\frac{0.059}{\alpha n_a}\right) Z_{H^+}$$

$$S_2 = S_1 Z_{H^+}$$

i.e.

$$Z_{H^+} = \frac{S_2}{S_1}$$

The estimated results indicated that, one proton ($Z_{H^+} = 1$) was participated in the rate-determining step of each of the reactant centers, and the number of electrons n_a are double that of protons Z_{H^+} (i.e. $Z_{H^+}/n_a = 0.5$). Values of the transfer coefficient α (Table 1) estimated either from S_1 ($\alpha = 59/S_1 n_a$) or S_2 ($\alpha = (59/S_2)(Z_{H^+}/n_a)$) confirmed the irreversible nature of the reduction processes of the drug at the mercury electrode. Accordingly, the general sequence of each of the irreversible reduction processes via the different waves of the reactant may be suggested as: H^+, e, e, $H^+_{(fast)}$ [44].

Table 1
Polarographic data for 2.5×10^{-4} M chlordiazepoxide in B–R buffers of different pH values, at 25 °C

pH	S_1 (mV)	αn_a	α[a] ($n_a = 2$)	S_2 (mV)	Z_{H^+}	α[b] ($Z_{H^+}/n_a = 0.5$)
First wave						
2.9	75.7	0.8	0.40	77.2	1.0	0.38
4.9	65.1	0.9	0.45		1.2	
6.9	64.7	0.9	0.45		1.2	
9.0	66.6	0.9	0.45		1.2	
Second wave						
2.9	48.9	1.2	0.60	57.4	1.2	0.51
4.9	49.1	1.2	0.60		1.2	
6.9	48.0	1.2	0.60		1.2	
9.0	48.7	1.2	0.60		1.2	
Third wave						
2.9	58.1	1.0	0.50	61.6	1.1	0.48
4.9	53.4	1.1	0.55		1.2	
6.9	52.9	1.1	0.55		1.2	
9.0	58.2	1.0	0.50		1.1	

S_1: slope of E_{de}–log$(i/i_d - i)$ plots, S_2: slope of $E_{1/2}$–pH plots.
[a] $\alpha = 59/S_1 n_a$.
[b] $\alpha = (59/S_2)(Z_{H^+}/n_a)$.

3.2. Cyclic voltammetric studies

The cyclic voltammograms of chlordiazepoxide at the hanging mercury drop electrode, in B–R buffers of pH 2–10 displayed three irreversible cathodic peaks, while a single irreversible cathodic peak was obtained at pH > 10, which are in accordance with the polarographic behavior. The peak potentials (E_p) shifted to more negative values upon rise of each of the pH (2–11) and the scan rate ν (20–500 mV s^{-1}) which, respectively, confirmed the involvement of protons in the electrode reaction and the irreversible nature of the reduction processes.

The E_p versus log(ν) plots at the different pH values were straight lines of slope values proportional to αn_a, from which it was found that two electrons (n_a = 2) were transferred in the rate-determining step. The peak current dependence of the three peaks upon the square root of scan rate suggested that the electrode process was mainly controlled by diffusion. The plots of log i_p (μA), of the three peaks in B–R buffers of pH 2–11, versus log ν were straight lines with slope values ranging from 0.58 to 0.52, which are close to the theoretical value of 0.5 that expected for ideal reaction of solution species [46]. So the reduction processes of chlordiazepoxide at the HMDE had mainly a diffusive component.

The interfacial adsorptive character of the drug onto the HMDE was identified from the peak current i_p (μA) dependence, after preconcentration of the drug for 30 s in B–R buffer of pH 8, upon the scan rate. The plots of log i_p versus log ν, gave a straight line followed the equation: $\log i_p(\mu A) = 0.94 \log \nu(\text{mV s}^{-1}) - 1.7$. The slope value of 0.94 is close to the theoretical value of 1.0 that expected for an ideal reaction of surface species [46]. The experimentally less slope value than the theoretical one, may be attributed to the partial involvement of the diffusive drug molecules in the electrode reaction of the adsorbed ones.

The interfacial adsorptive character of the drug was also identified from the voltammograms for 3×10^{-6} M chlordiazepoxide in B–R buffer of pH 8 recorded at scan rate of 200 mV s^{-1} without preconcentration (Fig. 3a) or followed preconcentration for 30 s (Fig. 3b). Followed preconcentration of the drug, three peaks were obtained, the second peak was much more developed and sharp (Fig. 3b). This behavior was attributed to the accumulation of the drug onto the HMDE surface during the preconcentration step. A substantial decrease of the cathodic peak currents was observed in subsequent scan at the same mercury drop (Fig. 3c) which indicates rapid desorption of the drug from the mercury surface.

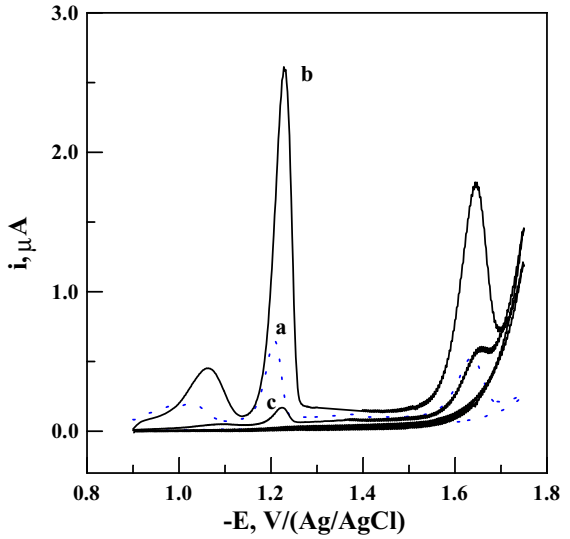


Fig. 3. Cyclic voltammograms for 3×10^{-6} M chlordiazepoxide in B–R buffer of pH 8 at scan rate = 200 mV s^{-1}: (a) without preconcentration, $t_{acc.}$ = 0 s (b) after preconcentration for 30 s at $E_{acc.}$ = −0.9 V, and (c) its repetitive cycle at the same mercury drop.

3.3. Stripping voltammetric studies

The square-wave adsorptive cathodic stripping (SWAdCS) voltammograms for 5×10^{-7} M chlordiazepoxide in B–R buffers of pH 2–11 followed preconcentration onto the HMDE for 30 s exhibited three irreversible cathodic peaks over the pH range 4–8.5, while in solutions of lower or higher pH values only one peak was obtained (Fig. 4). The second peak was much more developed and sharp at pH 8–8.5, so pH 8 was chosen for the rest of the analytical study.

3.3.1. Optimization of the operational parameters

Variation of the stripping voltammetric peak current of chlordiazepoxide in B–R buffer of pH 8 at the HMDE with instrumental variables such as accumulation time, accumulation potential, pulse-amplitude, scan increment and frequency were investigated using the square-wave mode.

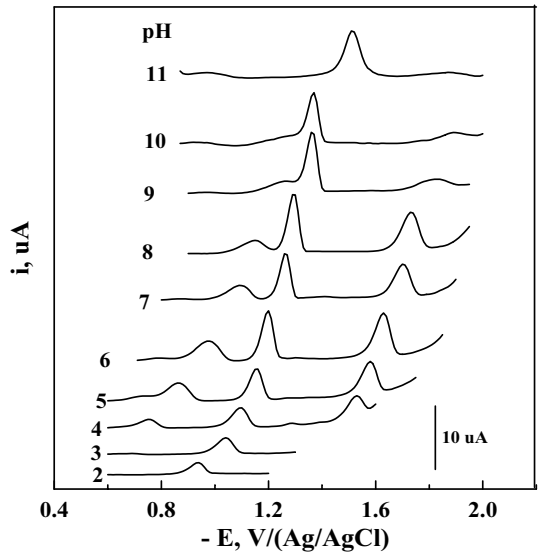


Fig. 4. SWAdCS voltammograms for 5×10^{-7} M chlordiazepoxide in B–R buffers of different pH values (2–11); $t_{acc.} = 30$ s, $E_{acc.} = -0.9$ V, scan increment = 10 mV, frequency = 120 Hz and pulse-amplitude = 50 mV.

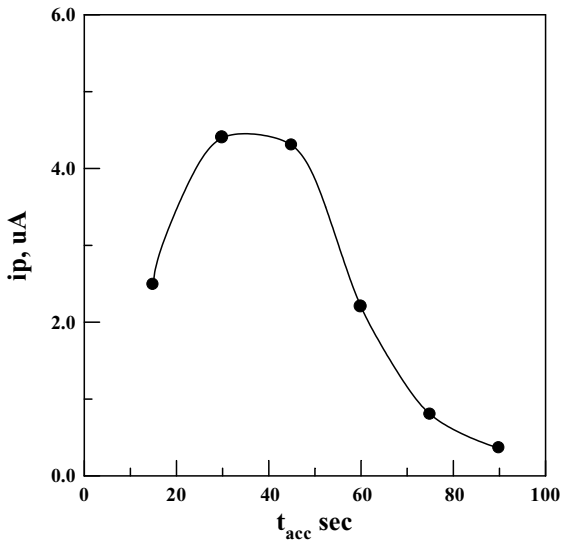


Fig. 5. Effect of accumulation time ($t_{acc.}$) on the SWAdCS voltammetry second peak current for 2×10^{-7} M chlordiazepoxide in B–R buffer of pH 8; $E_{acc.} = -0.9$ V. Other operational conditions are as those indicated in Fig. 4.

The influence of accumulation potential on the second peak current of chlordiazepoxide was examined in B–R buffer of pH 8 over the range −0.1 to −0.9 V. The results showed no obvious effect of $E_{acc.}$ on the peak current within the potential range studied. So, a potential of −0.9 V was chosen for the rest of the study.

The effect of accumulation time on the SWAdCS voltammetry second peak current of the drug in B–R buffer of pH 8, was investigated. An equilibrium surface concentration was reached at $t_{acc.}$ = 30–40 s (Fig. 5). At longer time periods, the peak current decreased which may be due to the complete coverage of the electrode surface with the drug species. A 30–40 s preconcentration period was therefore chosen for the rest of the study.

Study of the effect of scan increment on the square-wave stripping second peak current of the drug in B–R buffer of pH 8 revealed that the peak current enhanced upon the increase of scan increment (2–10 mV). A scan increment of 10 mV was preferable in the present study. At pulse-amplitude of 50 mV, the second peak current was found to be much more sharp and defined. Moreover, the peak current of the drug was found to increase linearly upon the increase of frequency f (20–120 Hz). A frequency of 120 Hz was chosen for the present analytical study.

The influence of the surface area of the working mercury electrode on the peak current was also studied. As expected, an increase of the electrode surface area generated a higher peak current, so a mercury drop of a large area (0.026 cm^2) was considered in the present study. The influence of the rest time was also considered and a time period of 5 s was chosen. Accordingly, the optimized operational conditions of the proposed stripping procedure were: B–R buffer at pH 8, $E_{acc.} = -0.9$ V, $t_{acc.}$ = 30–40 s, frequency = 120 Hz, scan increment = 10 mV, pulse-amplitude = 50 mV, stirring rate = 400 rpm, rest time = 5 s and size of the mercury drop = 0.026 cm^2.

3.3.2. Validation of the procedure

3.3.2.1. Linearity. The applicability of the proposed square-wave adsorptive cathodic stripping voltammetric procedure as an analytical method for the determination of chlordiazepoxide was examined by measuring the stripping peak current as a function of concentration of the bulk drug for at least three times under the optimized operational parameters. Three calibration graphs were constructed.

The dependency existed between peak current and the concentration of the drug was rectilinear within the range 5×10^{-9} M to 2×10^{-7} M. The calibration graph was represented by the equation: $i_p(\mu A) = (20.65 \pm 0.15)\,C\,(\mu M) + (0.057 \pm 0.003)$; ($r = 0.998 \pm 0.001$ and $n = 16$). The limits of detection (LOD) and quantitation (LOQ) of the drug were calculated using the following equations [47]:

$$\mathrm{LOD} = \frac{3s}{m}$$

and

$$\mathrm{LOQ} = \frac{10s}{m}$$

where s is the standard deviation of the intercept and m is the slope of the calibration graph. The estimated values of LOD and LOQ of bulk chlordiazepoxide were found to be 4.4×10^{-10} M and 1.5×10^{-9} M, respectively.

3.3.2.2. Specificity. The specificity of the optimized procedure for the assay of chlordiazepoxide was identified by study of the effect of various substances that oven accompany the drug in various pharmaceutical preparations. An attractive feature of an analytical procedure is its relative freedom from interference by the usual excipients such as cellulose, magnesium stearate, glucose, lactose, dextrose and starch. Samples containing 7×10^{-8} M bulk chlordiazepoxide and different concentrations of the excipient under evaluation were analyzed by means of the proposed procedure. The obtained mean percentage recoveries (%R) and the relative standard deviations (%R.S.D.), based on the average of five replicate measurements (99.6 ± 0.5 to 101.3 ± 0.8) showed that no significant interference from excipients. On the other hand, the co-formulated clidinium bromide compound was found to be electro-inactive at the mercury electrode under the present conditions of investigation. Thus, the proposed procedure was able to assay of chlordiazepoxide in the presence of the excipients and/or the co-formulated clidinium bromide compound, and it can be considered specific or selective.

3.3.2.3. Accuracy. The accuracy of the analysis, using the proposed procedure, was identified by calculating systemic error involved. It was determined by estimating the recoveries of 1×10^{-8} M and 5×10^{-8} M bulk drug using the calibration curve and standard addition method. The estimated mean percentage recoveries based on five replicate measurements were 99.7 ± 0.6 and 99.7 ± 0.9, respectively, which indicates the high accuracy of the proposed analysis procedure.

3.3.2.4. Precision. The intra-day and inter-day precision of the proposed procedure was estimated by analyzing 5×10^{-8} M and 1×10^{-7} M bulk chlordiazepoxide solutions for three times in 1 and 5 days over a period of 1 week. The intra-day and inter-day coefficients of variation, for the recovery of the studied concentrations varied from 0.4 to 0.5 and 0.6–1.2, respectively. The results confirmed both the good precision of the proposed procedure and stability of the drug's solutions.

3.3.2.5. Repeatability. The repeatability [48] was examined by performing five replicate measurements for 5×10^{-8} M bulk drug followed preconcentration for 30 s under the same operational conditions. Percentage recoveries (%R) of 99.2, 99.8, 100.2, 100.1 and 98.9 were achieved with a mean value of 99.6 and %R.S.D. of 0.5, which indicates repeatability and high precision of the proposed procedure.

3.3.2.6. Robustness. The robustness [48], as a measure of procedure· s capability to remain unaffected by small variations in the operational parameters, was examined by analysis of 5×10^{-8} M bulk drug under the successive variation of: pH 8–8.5, accumulation potential $E_{acc.} = -0.8$–0.9 V, preconcentration period $t_{acc.} = 30$–35 s or pulse-amplitude = 50–55 mV. The obtained mean percentage recoveries (Table 3) based on the average of five replicate measurements were not significantly affected within the studied range of variations of some operational parameters, and consequently the proposed procedure can be considered robust.

3.3.2.7. Ruggedness. The ruggedness [48] of the measurements was examined by assay of 5×10^{-8} M bulk chlordiazepoxide using two potentiostats models 394 and 263A-PAR, under the same optimized operational conditions at different elapsed time. The mean percentage recoveries obtained due to Lab (1) to (2) variation were found reproducible, since there was

Table 2
Influence of variation of some of the operational parameters of the proposed procedure on the mean percentage recovery of 1×10^{-8} M bulk chlordiazepoxide; frequency = 120 Hz and scan increment = 10 mV

Parameter	%R ± S.D.[a]	Condition
pH of the medium[b]		
8	99.6 ± 0.5	$E_{acc.}$ = −0.9 V, $t_{acc.}$ = 30 s, a = 50 mV
8.5	98.7 ± 0.9	
Preconcentration potential, $E_{acc.}$[b] (V)		
−0.8	99.4 ± 0.7	pH = 8, $t_{acc.}$ = 30 s, a = 50 mV
−0.9	99.6 ± 0.5	
Preconcentration time, $t_{acc.}$[b] (s)		
30	99.6 ± 0.5	pH = 8, $E_{acc.}$ = −0.9 V, a = 50 mV
35	101.9 ± 1.1	
Pulse-amplitude, a[b] (mV)		
50	99.6 ± 0.5	pH = 8, $E_{acc.}$ = −0.9 V, $t_{acc.}$ = 30 s
60	100.8 ± 0.7	
Potentiostat (PAR)		
Model 394, Lab (1)	99.6 ± 0.5	pH = 8, $E_{acc.}$ = −0.9 V, $t_{acc.}$ = 30 s, a = 50 mV
Model 263 A, Lab (2)	99.3 ± 0.9	

[a] Average of five replicate measurements.
[b] Using Potentiostat 394.

no significant difference between the %R and R.S.D. values (Table 2).

3.4. Applications

3.4.1. Assay of tablets

The proposed procedure was applied to the analysis of Librax tablets. The precision was estimated for 3–11 ng of the drug using the calibration graph and standard addition method. The obtained mean percentage recoveries (%R) and the relative standard deviations (%R.S.D.) based on the average of five replicate measurements were found to be 98.3–100.9 and 0.8–1.1, respectively (Table 3). The procedure did not require any time-consuming extraction or evaporation steps prior to the assay of the drug. The obtained results were favorably compared to those obtained by a reported spectrophotometric method [4].

Table 3
Quantification of chlordiazepoxide in Librax tablets by the proposed procedure

Concentration added[a] (ng)	Concentration found[a] (ng)	%R	%R.S.D.
3.0	2.95	98.3	0.8
5.0	4.93	98.6	0.8
8.0	8.07	100.9	1.0
11.0	11.10	100.9	1.1

[a] Average of five replicate measurements.

3.4.2. Assay of spiked human serum

The proposed procedure was also applied to the determination of different concentrations of the drug spiked in human serum after deproteinization with ethanol. Representative voltammograms are shown in Fig. 6. The linear variations of SW stripping peak current with concentration of the drug spiked in human serum within the range 5×10^{-9} M to 2×10^{-7} M was measured three times and three corresponding calibration graphs were obtained. The calibration graph was represented by the equation: $i_p(\mu A) = (13.6 \pm 0.2)\,C\,(\mu M) + (0.167 \pm 0.003)$; ($r = 0.997 \pm 0.001$ and $n = 10$). The limits of detection and quantitation of chlordiazepoxide spiked in human serum estimated by means of the proposed procedure was found to be 6.6×10^{-10} M and 2.2×10^{-9} M, respectively. The procedure did not require sample pretreatment or time-consuming extraction or evaporation steps, other than deproteinization of serum samples with ethanol, then the centrifugal separation of the precipitated protein, prior to analysis of the drug. The precision

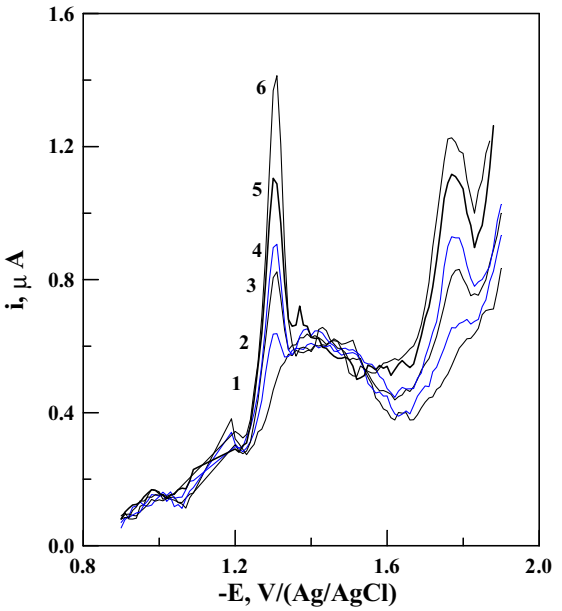


Fig. 6. SWAdCS voltammograms for different concentrations of chlordiazepoxide spiked in human serum: (1) background; (2) 5×10^{-9} M; (3) 1.5×10^{-8} M; (4) 2.0×10^{-8} M; (5) 3.5×10^{-8} M and (6) 5×10^{-8} M; B–R buffer of pH 8.0; $t_{acc.} = 30$ s, and $E_{acc.} = -0.9$ V. Other operational conditions are as those indicated in Fig. 4.

Table 4
Quantification of chlordiazepoxide spiked in human serum samples by the proposed procedure

Concentration added[a] (ng)	Concentration found[a] (ng)	%*R*	%R.S.D.
3.0	3.03	101.0	0.6
5.0	5.05	101.0	0.7
8.0	7.08	101.1	1.0
10.0	10.11	101.1	1.3

[a] Average of five replicate measurements.

of the analysis was estimated from five replicate measurements for 3–10 ng of the drug spiked in human serum. The mean percentage recoveries (%*R*) and the relative standard deviations (%R.S.D.) based on the average of five replicate measurements were found as 101.0–101.1 and 0.6–1.3, respectively (Table 4).

3.5. Conclusion

The electrode reaction pathway of chlordiazepoxide at the mercury electrode in B–R buffers of pH 2–11 has been elucidated. A validated square-wave adsorptive cathodic stripping voltammetric procedure has been described to the quantitation of the drug in bulk form, tablets and human serum. The procedure did not require sample pretreatment or time-consuming extraction or evaporation steps prior to analysis of the drug. The proposed procedure is much more simple, fast, sensitive, selective, costly low and achieved much more lower limits of detection and quantitation compared to those obtained by previously reported methods.

Acknowledgements

The authors express their gratitude to the Alexander von Humboldt Foundation (Bonn, Germany) for donation of the Electrochemical Trace Analyzer 263A-PAR, the Eppendorf centrifuge 5417C and the personal computer used in the present work to Prof. Dr. Mohamed Ghoneim. In addition, all the other facilities provided from Dr. M. Ghoneim's research laboratory of electrochemistry (Chemistry Department, Faculty of Science, Tanta University) to carry out the measurements of this work are also appreciated.

References

[1] G.K. Mcevory (Ed.), AHFS Drug Information, American Society of Hospital Pharmacists, 1990, pp. 1256–1258.
[2] J. Gasparic, J. Zimak, J. Pharm. Biomed. Anal. 1 (1983) 259–279.
[3] B.C. Rudy, B.Z. Senkowski, in: K. Florey (Ed.), Analytical Profiles of Drug Substance, vol. 2, Academic Press, New York, 1973, pp. 145–161.
[4] M.I. Toral, P. Richter, N. Lara, P. Jaque, C. Soto, M. Saavedra, Int. J. Pharm. 189 (1999) 67–74.
[5] S.A. Ozkan, E.N. Senturk, Anal. Lett. 32 (1999) 497–520.
[6] A. Elbrashy, F.A. Aly, F. Belal, Microchim. Acta 110 (1993) 55–60.
[7] M.I. Walash, M. Rizk, A. Elbrashy, Talanta 35 (1988) 895–898.
[8] M. Bedair, M.A. Korany, M.E. Abdelhamid, Analyst 109 (1984) 1423–1426.
[9] B.A. Koechlin, L. D'Arconte, Anal. Biochem. 5 (1963) 195–202.
[10] B.A. Koechlin, M.A. Schwatrz, G. Krol, W. Oberhansli, J. Pharmacol. Exp. Ther. 148 (1965) 399–403.
[11] E. Tanaka, M. Terada, S. Misawa, C. Wakasugi, J. Chromatogr. B 709 (1998) 324–334.
[12] E. Tanaka, M. Terada, S. Misawa, C. Wakasugi, J. Chromatogr. B 682 (1996) 173–178.

[13] V. Soentjenswerts, J.G. Dubois, G. Atassi, M. Hanocq, J. Chromatogr. A. 662 (1994) 255–262.
[14] M.A. Abuirjeie, M.E. Abdelhamid, Anal. Lett. 22 (1989) 951–962.
[15] I.M. Jalal, S.I. Sasa, A. Hussein, Anal. Lett. 20 (1987) 635–655.
[16] S.E. Roberts, M.F. Delaney, J. Chromatogr. 283 (1984) 265–272.
[17] T.B. Vree, A.M. Baars, Y.A. Hekster, E. Vanderkleijn, J. Chromatogr. 224 (1981) 519–525.
[18] R. Saelzer, G. Godoy, M. Vega, M. de Diego, R. Godoy, G. Rios, J. AOAC Int. 84 (2001) 1287–1295.
[19] C. Sarbu, G. Cimpan, J. Planar Chromatogr.-Modern TLC 9 (1996) 126–128.
[20] S.M. Yuen, G. Lehr, J. Associat. Offic. Anal. Chemists 74 (1991) 461–464.
[21] M. Divoll, D.J. Greenblatt, R.I. Shader, Pharmacology 24 (1982) 261–266.
[22] R. Montero, M. Gallego, M. Valcarcel, Analyst 115 (1990) 943–945.
[23] W.F. Smyth, Voltammetric Determination of Molecules of Biological Significance, Wiley, Chichester, 1992.
[24] M.M.C. dos Santos, V. Famila, M.L.S. Goncalves, Anal. Biomed. Chem. 374 (2002) 1074–1081.
[25] H. Oelschlager, Arch. Pharm. 296 (1963) 396–401.
[26] B.Z. Senkowsk, M.S. Levin, J.R. Urbigkit, E.G. Wollish, Anal. Chem. 86 (1964) 1991–1994.
[27] H. Oelschlager, H. Hoffman, Arch. Pharm. 300 (1967) 817.
[28] H. Oelschlager, H. Hoffman, E. Kurek, Arch. Pharm. 300 (1967) 250.
[29] J.M. Clifford, W.F. Smyth, Fresenius Z. Anal. Chem. 264 (1973) 149.
[30] E. Jacobsen, T.V. Jacobsen, Anal. Chim. Acta 55 (1971) 293–301.
[31] W. Guo, H. Lin, L.M. Liu, Z.A. Guo, J.F. Song, Chin. J. Chem 20 (2002) 502–508.
[32] W. Lund, L.N. Opheim, Anal. Chim. Acta 88 (1977) 275–279.
[33] G. Caille, J. Braun, J.A. Mockle, Can. J. Pharm. Sci. 5 (1970) 78–81.
[34] G. Cimbura, R.C. Gupta, J. Forensic Sci. 10 (1965) 282–286.
[35] M.A. Brooks, J.A.F. de Silva, Talanta 22 (1975) 849–860.
[36] M.R. Hackman, M.A. Brooks, J.A.F. de Silva, Anal. Chem. 46 (1974) 1075–1082.
[37] E. Lorenzo, L. Hernandez, Anal. Chim. Acta 201 (1987) 275–280.
[38] C. Yarnitzky, W.F. Smyth, Int. J. Pharm. 75 (1991) 161–169.
[39] J. Wang, Analytical Electrochemistry, second ed., Wiley–VCH, New York, 2000, pp. 75–84.
[40] J. Osteryoung, J.J. O'Dea, in: A.J. Bard (Ed.), Electrochemistry Chemistry. A Series of Advances, vol. 14, Marcel Dekker, New York, p. 209.
[41] S. Borman, Anal. Chem. 54 (1982) 698A.
[42] Britton HTS Hydrogen Ions, fourth ed., Chapman & Hall, London, 1952, p. 113.
[43] L. Meits, Polarographic Techniques, second ed., Interscience, New York, 1965, p. 232.
[44] P. Zuman, The Elucidation of Organic Electrode Processes, Academic Press, New York, 1969, pp. 21–24.
[45] M.M. Ghoneim, M.A. Ashy, Can. J. Chem. 57 (1979) 1294–1298.
[46] E.J. Laviron, Electroanal. Chem. 112 (1980) 1–9.
[47] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, fourth ed., Ellis-Howood, New York, 1994, p. 115.
[48] The United States Pharmacopoeia, The National Formulary, USP 24, NF 19, USP Convention Inc., 2000.