Adsorptive voltammetric determination of chlordiazepoxide in pure and dosage forms

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Abstract

The behavior of chlordiazepoxide has been studied using dc-polarography at dropping mercury electrode (DME) and cyclic voltammetry at glassy carbon electrode (GCE). In Britton-Robinson buffers, the drug developed three reduction irreversible waves in acidic solutions, and only two waves in alkaline media. The adsorptive stripping voltammetric behavior of chlorodiazepoxide was studied at GCE, and a sensitive voltammetric method was evaluated applying linear-sweep mode for quantitative determination of the drug in pure form and commercial tablets. A linear relationship between peak current and drug concentration was obtained in the concentration range $2x10^{-7}$ to $5x10^{-6}$ mol l^{-1} of the drug at pH = 4.0, with a detection limit of $5x10^{-8}$ M. The drug was determined in capsules with a percentage recovery of 100.65 ± 1.83 (n=5).

Keywords: adsorptive; voltammetry; glassy carbon electrode; chlordiazepoxide.

Introductuion

Chlordiazepoxide, (Fig. 1) is [7-chloro-2-(methylamino)-5-phenyl-3H-1,4-benzodiazepine-4-oxide]. This drug may inhibit monosynaptic and polysynaptic reflexes by acting as inhibitory neutral transmitters or by blocking excitatory synaptic transmission. It may also directly depress motor nerve and muscle function [1,2] and anxiety-related conditions including spastic colon [3]. Chlordiazepoxide has been studied and determined by several procedures including spectrophotometry [4-6], derivative spectroscopy [7] and atomic absorption spectrometry (AAS) of its reduced form [8]. Many electrochemical procedures have been reported for the determination of chlordiazepoxide. The polarographic behavior of the drug at the mercury electrode has been studied in different media [9-12]. Chlordiazepoxide has been determined in different samples by different techniques as dc-polarography [13-15], differential-pulse polarography [16,17], differential-pulse stripping voltammetry [18] and square-wave voltammetry [19]. The voltammetric behavior and quantification of the drug has been carried out in bulk form, pharmaceutical formulation and serum onto the mercury electrode [20].

The aim of the present work is to investigate the electrochemical behavior of chlordiazepoxide at the glassy carbon electrode as a safe electrode material (in contrast to mercury electrodes) and to optimize the conditions for the determination of this compound in

pharmaceutical dosage forms and human serum using adsorptive linear-scan voltammetry technique.

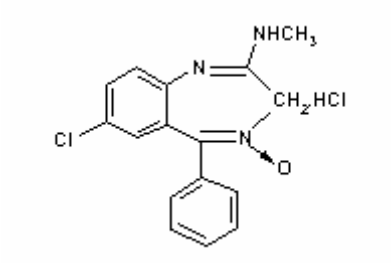

Fig 1. Molecular structure of chlordiazepoxide

Experimental Section

Apparatus

The dc polarographic study was carried out using a Sargent-Welch polarograph model 3001. The electrochemical cell was a glass beaker with two electrode: DME (m= 0.37, t= 3.6 s, h= 60 cm) as working electrode and SCE as a reference electrode. All measurements were carried out at room temperature.

Cyclic and linear-scan voltammograms were recorded by a potentiostat model 273 A with a software model HQ 2030. A three-electrode cell was equipped with a glassy carbon working electrode, a platinum-wire auxiliary electrode and an Ag/AgCl reference electrode. The solutions were purged with pure nitrogen gas for 5 min before being analyzed at room temperature.

Materials and reagents

Chlordiazepoxide was provided by EVA Pharmaceutical Company. All reagents were of analytical-reagent grade. De-ionized water was used throughout the experiments. Britton-Robinson buffer of pH range 2.0-12.0 was prepared by adjusting an acid solution mixture (0.4 M in each of the following acids: acetic acid, boric acid and ortho-phosphoric acid) with 0.8 M NaOH solution to the desired pH value. A standard drug solution ($1.00x10^{-3}$ mol l^{-1}) was prepared in ethanol then serially diluted with the same solvent as appropriate. The working solutions were prepared by adding suitable volumes of the stock to the buffer solution and adjusting the final percentage of ethanol at 20% (v/v).

Procedure

Procedure of standard chlordiazepoxide hydrochloride

Stock solutions of chlordiazepoxide hydrochloride ($1.00x10^{-3}$ mol l^{-1}) were prepared in pure ethanol and kept in refrigerator. The working solutions were prepared by diluting definite volumes with supporting electrolyte and aqueous/ethanol solution to obtain the desired concentrations.

Procedure for capsules

Weight the contents of 10 capsules of the commercial formulation of the drug (Cloxide®, EVA Pharm. Co.) which contain 5 mg/tablet. Transfer an accurately weighed quantity of the powder equivalent to 37.2 mg of chlordiazepoxide into a small beaker. Shake gently for 20 min with about 80 ml aqueous solution containing 20% ethanol and filter into a 100 ml measuring flask. Wash the beaker and the filter paper then transfer the washing into the same measuring flask. Complete to the mark with the same solvent to obtain $1.00x10^{-3}$ mol l^{-1} of the drug solution. Transfer aliquot volumes containing suitable amounts of the drug into

series of 25 ml measuring flasks and complete to the mark with the supporting electrolyte. Transfer the whole contents of the flask into the working cell. Pass pure nitrogen for 5 min before measurements. Record the current in negative direction from 0 to -1500 mV vs. reference electrode.

Procedure for serum

Serum samples obtained from healthy individuals were stored frozen until assay. After gentle thawing, an aliquot of sample was fortified with chlordiazepoxide dissolved in de-ionized water to achieve the appropriate concentration. The supernatant was taken carefully. An appropriate volumes of supernatant liquor was transferred to a 10 ml volumetric flask, diluted with buffer solution of pH 4.00 and 30% ethanol. The proposed method was used for assaying the drug.

Results and Discussion

DC polarography

The dc-polarograms of chlordiazepoxide in B-R buffers of pH values 2.0-7.8 exhibited three irreversible reduction waves of almost equal heights. In alkaline solutions (pH>8), only two waves were observed, Fig 2. The half-wave potentials of the three waves shifted to more negative potentials on increasing the pH of the working solutions indicating a proton-transfer reaction precedes the electrode process [21]. The plots of E_{de} vs log [i/(i_d-i)] for the three waves at different pH values gave straight lines of slope S_1=59/αn_a. From the values of αn_a, the value of the transfer coefficient (α) was calculated. These values of α (0.45-0.56) reflect the irreversible nature of the electrode process. The number of hydrogen ions (Z_{H+}) participating in the rate-determining step was calculated to be one from the values of the slopes S_1 (of E_d- log [i/(i_d-i)] plots) and S_2 (of $E_{1/2}$ – pH plots); where: (Z_{H+}) = S_2/S_1.

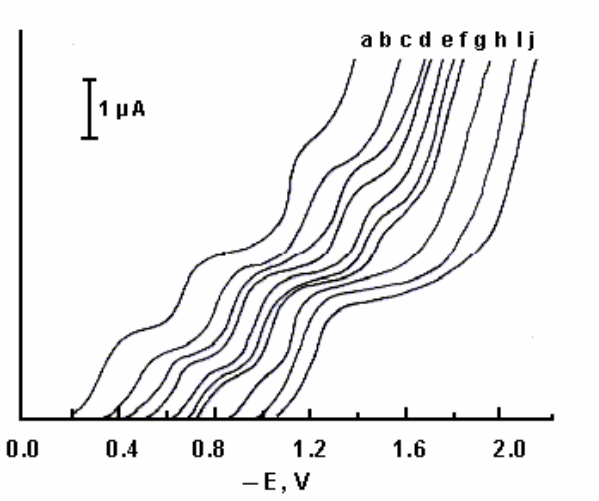

Fig 2. DC polarograms of 1x10^{-3} mol l^{-1} chlordiazepoxide in B-R buffer solutions of different pH values (a: 2.0, b: 3.1, c: 4.2, d: 5.1, e; 6.0, f: 7.0, g: 7.9, h: 9.8, i: 10.5, j: 11.8)

A controlled-potential coulometric analysis of a definite concentration of chlordiazepoxide at pH 5.0 using a large mercury pool as a cathode, indicated that two electrons were consumed in each of the three waves.

According to the data obtained from the reduction process and a previous work reported for the dc reduction of chlordiazepoxide, the reduction mechanism can be suggested as following:

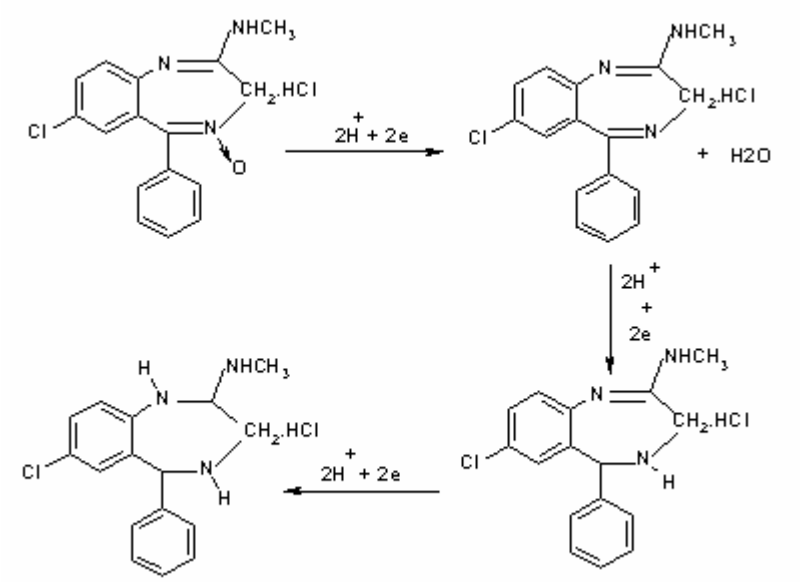

Fig 3. Electrode reaction mechanism of chlordiazepoxide

Cyclic voltammetry

Cyclic voltammetric behavior of chlordiazepoxide showed two irreversible reduction peaks, at about -4.0 and -7.5 V, in B-R buffers at acidic solutions (pH 2.0-6.5). The absence of any oxidation peak on the reverse scan indicates that the reduction is totally irreversible. The plot of peak potentials (E_p) of the two peaks versus pH shows a negative shift of peak potentials on increasing pH values. This behavior confirms the involvement of protons in the electrode reaction. The increase of the scan rate (υ) from 50 to 500 mVs^{-1} causes also a shift in E_p towards more negative values which is an indication of the irreversible nature of the electrode process. The E_p plots versus log υ at different pH values were straight lines of slopes proportional to αn_a, from which the value of n_a was calculated to be 2.02±0.05 (n=5).

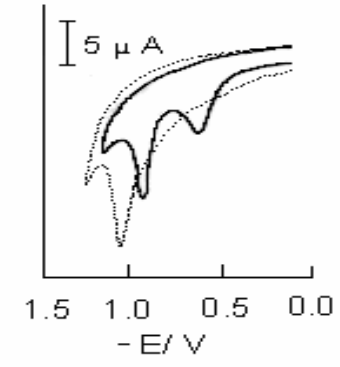

Fig 4. Cyclic voltammetry of $1x10^{-4}$ mol/L chlordiazepoxide at pH 2.15 (—) and 10.45 (···)

The influence of υ on the peak current i_p was studied in the range 50–500 mVs^{-1}, resulting in a linear relationship (r=0.996, n=6) between i_p and $\upsilon^{1/2}$ with a slope of 0.54. This value of slope indicates that the current is of diffusion nature [22]. The plot of log i_p versus log υ gave a straight line expressed by the equation:

Log i_p = 19.79 log υ – 32.75 (at pH 2.50) with a slope 0.93 which is too close to the theoretical value (1.0) expected for an ideal reaction of surface species [23].

Cathodic adsorptive stripping voltammetry(CAdSV)

Different parameters affecting the adsorptive stripping response of chlordiazepoxide at the glassy carbon electrode were examined to achieve high sensitivity for drug quantification. These parameters include various experimental and instrumental variables.

Effect of supporting electrolyte and pH

The cathodic adsorptive behavior of chlordiazepoxide was investigated in different supporting electrolytes (KCl, HCl, $NaClO_4$, acetate buffer, phosphate buffer and B-R buffer). Two reduction peaks were obtained in all cases, with the exception of KCl and $NaClO_4$ solutions, where only one peak has been appeared. The drug yielded sharp and well-developed peaks with B-R buffers of pH 4.0-7.5.

Effect of accumulation time and potential

Preconcentration of the analyte drug on the surface of GCE is an important condition for highly sensitive determinations. Variation of the accumulation time over 0.0-240 s was examined for $5.00x10^{-5}$ mol l^{-1} chlordiazepoxide solution at an accumulation potential of -0.4 V. The dependence of peak current (i_p) on accumulation time ($t_{acc.}$) is presented in Fig 5. The peak heights of the two peaks increases with increasing t_{acc} from 0.0 to 30 s, then, they decreased rapidly with increasing accumulation time. Thus, an accumulation time of 30 s was adopted as optimum operational value for analytical purposes.

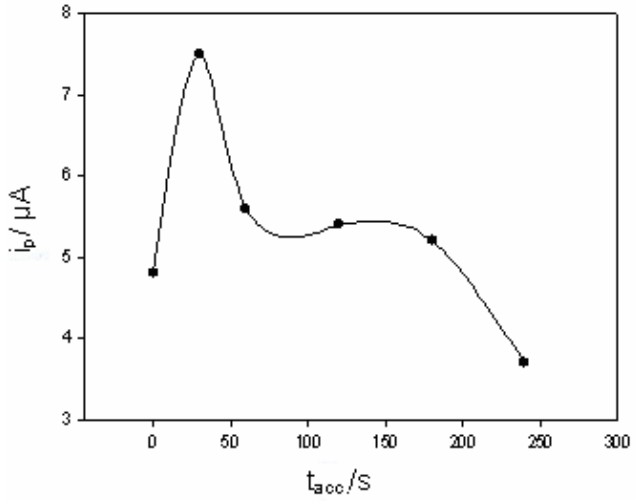

Fig 5. Effect of accumulation time of $5x10^{-5}$ mol l^{-1} chlordiazepoxide at pH 4.0 at $E_{acc.}$=-400 mV, ν=500 mVs^{-1}

In addition, as can be seen from Fig 6, the effect of accumulation potential (E_{acc}) on the peak height was examined over the potential range +0.1 to -0.8 V for the same concentration of the drug at accumulation time of 240 s. The peak current increased steadily over the negative direction till it reached its maximum value at E_{acc}= -0.4 V where it decreased gradually after this inflection point. According to this relationship, E_{acc}= -0.4 V is the optimum value for drug quantification.

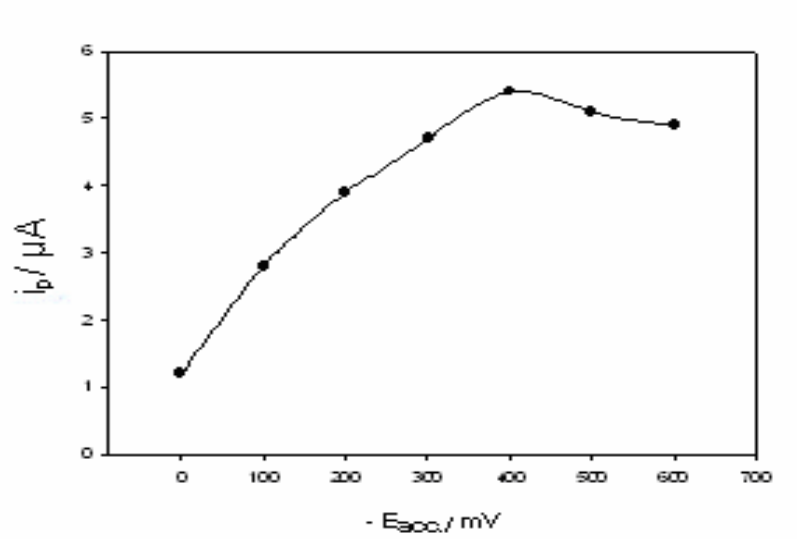

Fig 6. Effect of accumulation potential of $5x10^{-5}$ mol l^{-1} chlordiazepoxide at pH 4.0 at $t_{acc.}$= 100 s, ν=100 mVs^{-1}

Effect of scan rate

The two cathodic peak currents of chlordiazepoxide were found to be directly proportional to the scan rate (υ) in the range 50-500 mVs^{-1} for $5.00x10^{-5}$ mol l^{-1} in B-R buffer of pH 4.00. Accordingly, 500 mVs^{-1} scan rate was adopted as optimum condition for further voltammetric investigations.

Effect of step height

The effect of the step height on the CAdSV peak current of the drug was recorded at different values (10-150 mV) for $5x10^{-5}$ mol l^{-1} chlordiazepoxide. A well-defined peak was observed at 10 mV step height for pH's 4.00 and 7.35. On increasing step height, the peak current decreases until it became more ill-defined. So, the value of 10 mV was chosen as the best value for further voltammetric studies.

Effect of drug concentration

The influence of chlordiazepoxide concentration on the cathodic peak current (Fig. 7) was evaluated in order to obtain a significant analytical utility. The peak current of the first reduction peak at about -0.7 V in B-R buffer of pH 4.00 increased linearly as a function of drug concentration over the range $2.00x10^{-7}$ to $5.00x10^{-6}$ mol l^{-1} chlordiazepoxide.

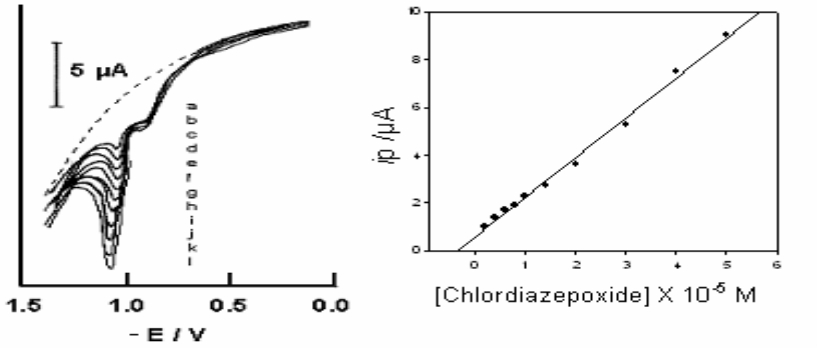

Fig 7. Effect of concentration of chlordiazepoxide on peak current at pH 4.0 at $t_{acc.}$= 30 s, $E_{acc.}$= -400 mV, ν=500 mVs^{-1}

(a: blank; b: 0.2; c: 0.5; d: 1.0; e: 1.5; f: 2.0; g: 2.5; h: 3.0; i: 3.5; j: 4.0;k: 4.5; l: $5.0x10^{-6}$ mol L^{-1})

Analytical applications

After the establishment of the most ideal conditions for the adsorptive determination of chlorodiazepoxide, a calibration plot for the analyzed drug was constructed to estimate the analytical characteristics of the developed method.

Calibration graph

Plots representing the relationship between the concentration of chlordiazepoxide and CAdSV peaks gave straight lines over the concentration range of 0.067-1.68 µg/ml ($2.00x10^{-7}$-$5.00x10^{-6}$ mol l^{-1}). Linear regression analysis of the data gave the following equation:

$$i_p\ (\mu A) = 1.57 + 1.46\ 10^6\ C\ (M);\ r = 0.9982\ \ (n=10)$$

Where i_p is the adsorptive stripping peak current, C the analyzed drug concentration, r the correlation coefficient and n is the number of points.

Detection limit

The lowest detectable concentration of the drug was evaluated to be 0.0168 µg/l ($5.00x10^{-8}$ mol l^{-1}) estimated according to the signal-to-noise ratio (S/N=3) of the response of $4.00x10^{-7}$ mol l^{-1} chlordiazepoxide. Such remarkable low value of detection limit demonstrates the high sensitivity of the current method.

Reproducibility

The high sensitivity of the adsorptive voltammetry is accompanied by very good reproducibility. The reproducibility was evaluated from 6 repeated measurements of the electrochemical signal of $2.00x10^{-6}$ mol l^{-1} chlordiazepoxide solution. The precision of the developed method in terms of the relative standard deviation (R.S.D.%) was 1.03%.

Determination of chlordiazepoxide in dosage forms

The reliability of the proposed method for determination of chlordiazepoxide was investigated by assaying the drug in some real samples (capsules), applying standard addition method. The results obtained are summarized in Table 1.

Table1. Adsorptive voltammetric assay of chlordiazepoxide in pharmaceutical formulation

Drug	Value Labeled (mg)	Found [a] (mg)	Recovery (%)	R.S.D. (%) [b]
Cloxide®	5	4.96	99.40	1.73
Librium®	10	10.13	101.20	2.15
Librium®	20	20.22	101.35	1.62

a: average of five determinations, b: relative standard deviation

Determination of chlorodiazepoxide in human serum

The ability of the proposed method for detection of the drug in human serum was examined in spiked serum samples. The results are summarized in Table 2.

Table 2. Adsorptive stripping determination of chlordiazepoxide in spiked serum

Added (mol l^{-1})	Found[a] (mol l^{-1})	Recovery (%)	R.D.S. (%)
$4.0x10^{-7}$	$3.88x10^{-7}$	97.00 %	1.77
$8.0x10^{-7}$	$7.93x10^{-7}$	99.12%	2.62
$1.2x10^{-6}$	$1.23x10^{-6}$	102.50%	2.84

a: average of five determinations

Conclusion

The voltammetric techniques proved that they are excellent alternative for the study of the reaction mechanisms and analytical determinations of drugs, especially nitro compounds. The proposed linear sweep voltammetry procedure can be used successfully to determine chlordiazepoxide in tablet dosage form and in human serum so, it can be applied for the analytical determination of chlordiazepoxide because it is simple, fast and low cost, and has sufficient precision, accuracy and sensitivity.

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