Notes

Study and Application of Polarographic Catalytic Wave of Chlordiazepoxide in the Presence of Persulfate

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Polarographic catalytic wave of chlordiazepoxide in the presence of $K_2S_2O_8$ was studied in aqueous and DMF/H_2O mixed solutions. The results showed that a single reduction wave in alkaline medium was the reduction of the N = C bond in 1,2-position of chlordiazepoxide via an intermediate free radical in two one-electron successive additions. When $K_2S_2O_8$ was present, the free radical of the N = C bond was oxidized to regenerate the original, producing a parallel catalytic wave of chlordiazepoxide. It was determined that the apparent rate constant k_f of the oxidation reaction was 3.2×10^3 mol^{-1}·L·s^{-1}. Using the catalytic wave the trace of chlordiazepoxide can be determined by linear-potential scan polarography. In NH_3/NH_4Cl (pH 10.2 ± 0.1, 0.12 mol/L)/$K_2S_2O_8$ (0.016 mol/L) supporting electrolyte, the second-order derivative peak current of the catalytic wave was rectilinear to chlordiazepoxide concentration in the range of 3.20×10^{-8}—1.60×10^{-7}, 1.60×10^{-7}—1.44×10^{-6} and 1.44×10^{-6}—1.44×10^{-5} mol/L, respectively. The limit of detection was 9.0×10^{-9} mol/L.

Keywords chlordiazepoxide, persulfate, polarographic catalytic wave, free radical

Introduction

Chlordiazepoxide (7-chloro-2-methylamino-5-phenyl-3*H*-1, 4-benzodiazepine-4-oxide) showing powerful antianxiety effect has been widely used as a psychotherapeutic drug. Consequently, the need arose for sensitive and rapid determination of chlordiazepoxide in blood, urine and pharmaceutical preparations. Spectrophotometric,[1] HPLC,[2] GC-FID[3] and voltammetric[4,5] methods for the determination of chlordiazepoxide were reported. Among them, using adsorptive stripping voltammetry (AdSV)[4] and differential plus polarography (DPP),[5] high analytical sensitivity can be achieved, based on polarographic reduction wave of chlordiazepoxide. However, they were only limited to the analytical purpose. The polarographic catalytic wave is a convenient technique for studying the kinetic of the intermediate of reactant reduction and for improving the analytical sensitivity. Besides polarographic catalytic wave of transition metal ions, polarographic catalytic wave of organic compound as catalyst in the presence of oxidant has received considerable attention. In such polarographic catalytic waves, the reducible groups were reduced to the corresponding intermediate free radical and were regenerated through oxidation reaction of the free radical by oxidant. Therefore, the polarographic catalytic wave of organic compound is a useful tool to study chemical properties and kinetics of free radical of organic compound under various chemical conditions, too. The polarographic catalytic waves of some classes of organic compounds, such as α,β-unsaturated carbonyl compound,[6,7] steride,[8] protein containing disulfate linkage[9,10] and pyridine derivative[11] in the presence of H_2O_2 or KIO_3 have been reported. However, the polarographic catalytic wave of chlordiazepoxide, one of 1,4-benzodiazepines, in the presence of oxidant has never been reported in the literature.

A polarographic catalytic wave of chlordiazepoxide in NH_3/NH_4Cl (pH 10.2 ± 0.1, 0.12 mol/L) buffer con-

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taining $K_2S_2O_8$(0.016 mol/L) was observed. The aim of the present work was to study reductive and catalytic processes of chlordiazepoxide and to propose a new method for the determination of chlordiazepoxide.

Experimental

Reagents

Chlordiazepoxide (0.01 mol/L) standard stock solution was prepared by dissolving 0.2998 g of chlordiazepoxide (Xi'an Pharmaceutical Factory) with 10 mL of HCl (1.0 mol/L) and diluting up to 100 mL with water. Chlordiazepoxide standard working solution was obtained by diluting the stock solution with water. Tablets were purchased from Xi'an Psychopathic Hospital with labeled amount 10 mg/tablet. All chemicals were of analytical grade. Twice distilled water was used through the experiments.

Apparatus

A model JP-3 linear-potential scan polarograph (Shandong Electric & Telecommunication Factory No. 7, China) was used. The three-electrode system involved a dropping mercury working electrode (DME), a platinum wire counter electrode and a saturated calomel reference electrode (SCE). The potential scan rate v was 0.25 V/s. The drop time of DME was 7 s. The mercury flow velocity was 0.437 mg/s. The area of the mercury drop was 4.44×10^{-3} cm^2. A model CH660 electrochemical workstation (CH Instrument, USA) was controlled by a CH660 software and worked under Windows environment. The three-electrode system involved a model 303A stationary mercury drop working electrode (EG&G PARC, USA), a platinum wire counter electrode and a SCE reference electrode. The area of the mercury drop was 4.1×10^{-3} cm^2.

An HP 1100 series liquid chromatogrph (Agilent Co., USA) equiped with a diode array detector was used. Chromatogrphic column was Zorbax C 8, 150 mm × 4.6 mm. An HP Chemstation achieved instrument control and data acquisition.

Procedure

Cyclic voltammograms of chlordiazepoxide in NH_3/NH_4Cl (pH 10.2 ± 0.1, 0.12 mol/L) buffer aqueous solution and in N,N-dimethylformamide (DMF)/H_2O mixed solution containing tetraethylammonium bromide [$(C_2H_5)_4NBr$] with or without $K_2S_2O_8$ were recorded on the model CH660 workstation. Dissolved oxygen was removed from the solution by passage of oxygen-free nitrogen gas for 10 min.

Results and discussion

Reduction process of chlordiazepoxide

Chlordiazepoxide has three reducible groups, a N-oxide and two N = C double bonds in 1,2 and 4,5 position of diazepine ring, respectively. Because the proton condition of medium largely influences the reduction process of organic compound,[12] chlordiazepoxide showed different polarographic behaviors in different media. In slightly acidic medium, the reduction of the N-oxide and the N = C double bond in 4,5 position of diazepine ring of chlordiazepoxide yielded two polarographic reduction waves. When pH was below 4.0, these two waves obtained were located at −0.26 V and −0.56 V, respectively.[13-15] In alkaline medium, the reduction of the N = C bond in 1,2 position of the diazepine ring produced a single reduction wave.[13,14] In this work, it was observed that the reduction wave could be catalyzed by $K_2S_2O_8$. To illustrate the polarographic catalytic wave of chlordiazepoxide, the reduction wave with peak potential −1.25 V was first examined in NH_3/NH_4Cl (pH 10.2 ± 0.1, 0.12 mol/L) buffer.

Cyclic voltammetry showed that there was a single reduction wave of chlordiazepoxide with peak potential −1.25 V on cathodic scan and no oxidation wave on reserve scan (Fig. 1, curve a). With potential scan rate v increasing from 0.25 to 10 V/s, the peak current I_{pc} of the reduction wave increased and the relationship between the I_{pc} and the v was

$$i_{pc}/\mu A = 0.1164 + 1.308v \quad (r = 0.9997,\ n = 8)$$

Moreover, the peak potential E_{pc} linearly shifted into negative direction with increasing the logarithm value ($\log v$) of the v. The relationship of E_{pc}-$\log v$ was

$$-E_{pc}/V = 1.282 + 0.0525\log v \quad (r = 0.9995,\ n = 8)$$

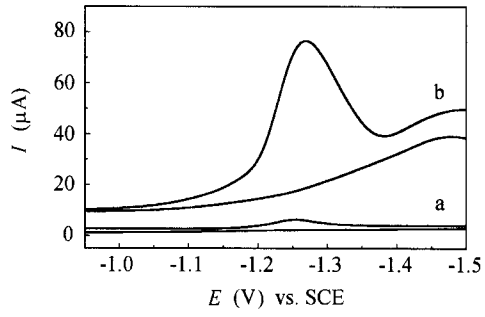


Fig. 1 Cyclic voltammograms of chlordiazepoxide (8.0×10^{-5} mol/L) in (a) NH_3/NH_4Cl (pH 10.2 ± 0.1, 0.12 mol/L) solution and (b) NH_3/NH_4Cl (pH 10.2 ± 0.1, 0.12 mol/L) + $K_2S_2O_8$ (0.016 mol/L) solution. Reverse scan from -1.50 V, scan rate 0.25 V/s.

Hence, the reduction wave of chlordiazepoxide was an irreversible one with adsorption characterisitics.[16]

The electron transfer number n for the single reduction wave was evaluated by means of the relationship of E_{pc}-log$v$. According to the linear-potential scan polarographic equation of irreversible process with adsorption of reactant

$$E_{pc} = E^0 + \frac{2.303RT}{\alpha nF}\left(\log\frac{RT}{\alpha nF}K^0 - \log v\right)$$

the n obtained was 2.1 from the slope value 0.0525 of the E_{pc}-log$v$ relationship (assuming electron transfer coefficient $\alpha = 0.5$). Furthermore, the E_{pc} shifted into negative direction with icreasing pH value. The E_{pc}-pH relationship was

$$-E_{pc}/V = 0.168 + 0.106\ pH \quad (r = 0.9992,\ n = 9)$$

The slope value of the E_{pc}-pH relationship demonstrated that two protons participated in the reduction process of the N = C bond.

To obtain detail information about reduction process of chlordiazepoxide, aprotic medium, DMF/H_2O mixed solution, was chosen. In 15 mL of DMF-$(C_2H_5)_4NBr$ (0.1 mol/L) medium mixed with 3.0 mL of H_2O [H_2O was added for the introduction of chlordiazepoxide standard solution (8.0×10^{-5} mol/L)], cyclic voltammetry showed that chlordiazepoxide yielded two reduction waves, P_1 and P_2, on cathodic scan and no oxidation wave on reserve scan (Fig. 2, curve a). The peak potential $E_{pc,1}$ of the wave P_1 was located at -1.32 V and the peak potential $E_{pc,2}$ of the wave P_2 at -1.62 V. Additionally, with raising H_2O amount in the DMF/H_2O mixed solution, both the $E_{pc,1}$ and the $E_{pc,2}$ shifted to positive direction, but the $E_{pc,2}$ shifted more quickly than the $E_{pc,1}$ did. At the same time, the peak current $I_{pc,1}$ of the wave P_1 increased and the peak current $I_{pc,2}$ of the wave P_2 decreased, while the sum $I_{pc,1} + I_{pc,2}$ of peak current of these two waves almost remained constant value (Fig. 2, curves b and c). When H_2O amount was up to 6 mL (Fig. 2, curve d), the two waves merged a single reduction wave with the peak potential -1.30 V, and the peak current of the single wave was equal to the $I_{pc,1} + I_{pc,2}$. The two reduction waves in DMF/H_2O mixed solution corresponded to the single reduction wave in aqueous solution. This indicated that the reduction process of the N = C bond in 1,2-position of chlordiazepoxide was two one-electron successive additions via an intermediate free radical. Because H_2O as the proton donor is present in the DMF/H_2O mixed solution, the reduction wave P_1 involves the reduction of the N = C bond to an intermediate free radical and the reduction of a part of the free radical to final product. The reduction wave P_2 is the further reduction of other part of the free radical. Therefore, the reduction process of the reduction wave of chlordiazepoxide can be described as shown in Scheme 1.

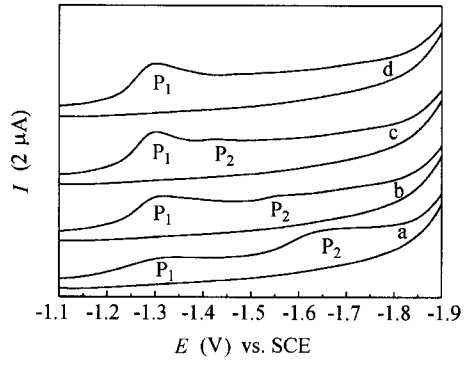
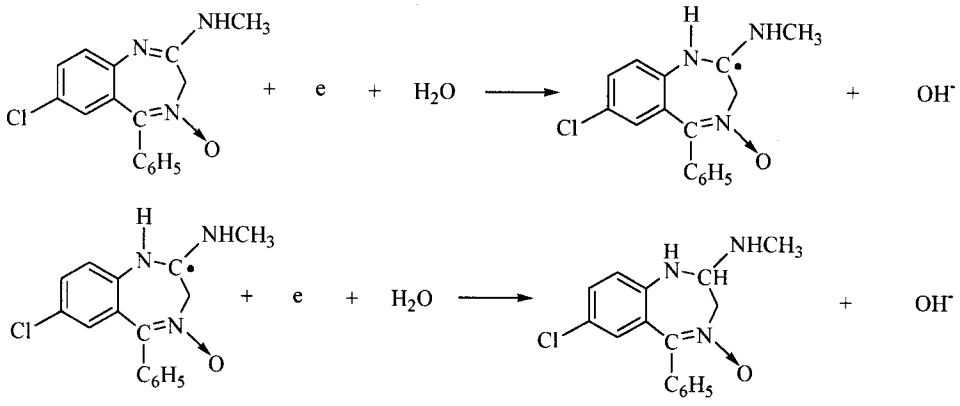


Fig. 2 Cyclic voltammograms of chlordiazepoxide (8.0×10^{-5} mol/L) in 15 mL of DMF/$(C_2H_5)_4NBr$ (0.1 mol/L) solution containing H_2O: (a) 3.0 mL, (b) 4.0 mL, (c) 5.0 mL and (d) 6.0 mL. Reverse scan from -1.90 V, scan rate 0.25 V/s.

Polarographic catalytic wave of chlordiazepoxide in the presence of $K_2S_2O_8$

When $K_2S_2O_8$ was present in NH_3/NH_4Cl (pH 10.2

Scheme 1



±0.1, 0.12 mol/L) buffer, cyclic voltammetry showed that the peak potential of the reduction wave of chlordiazepoxide on cathodic scan remained unchanged and the peak current increased greatly, no oxidation wave on reserve scan (Fig. 1, curve b). The enhanced wave was a polarographic catalytic wave.

The peak current I_{pk} of the catalytic wave increased with $K_2S_2O_8$ concentration increasing from 4.0×10^{-3} to 2.0×10^{-2} mol/L, and the ratio of I_{pk} to the peak current I_{pc} of the corresponding reduction wave was linearly proportional to square root of $K_2S_2O_8$ concentration. The relationship was

$$I_{pk}/I_{pc} = 166\ c_{K_2S_2O_8}^{1/2} \quad (r = 0.9948,\ n = 6)$$

Moreover, according to the current equation for both the reduction wave and the catalytic wave with adsorption characteristics in linear-potential scan polarography, the dependence of the peak current on the potential scan rate v is linear.[17] The current function was defined by $I_p \cdot v^{-1}$. The current function $I_{pk} \cdot v^{-1}$ of the catalytic wave at first decreased with the scan rate v increasing in the range of 50—300 mV/s, then leveled off with further raising the scan rate v (Fig. 3, curve b). While the function $I_{pc} \cdot v^{-1}$ of the corresponding reduction wave without $K_2S_2O_8$ almost remained constant value (Fig. 3, curve a). These facts illustrated that the catalytic wave of chlordiazepoxide in the presence of $K_2S_2O_8$ was a parallel one.

In the catalytic wave, chlordiazepoxide is a catalyst that can be reduced and regenerated. On the other hand, it is known that the $S_2O_8^{2-}$ is a strong oxidant and can oxidize the intermediate free radical of chlordiazepoxide to reproduce the original N = C bond, producing the parallel catalytic wave. Meanwhile, the $S_2O_8^{2-}$ was reduced via a sulfate radical $SO_4^{-\cdot}$ during the oxidation reaction. The sulfate radical $SO_4^{-\cdot}$ that was intermediate product of one-electron reduction of the $S_2O_8^{2-}$ was more active than the $S_2O_8^{2-}$ and also took part in the oxidation process. The oxidation reaction of the free radical of chlordiazepoxide by both the $S_2O_8^{2-}$ and the SO_4^{-} can be written as shown in Scheme 2.

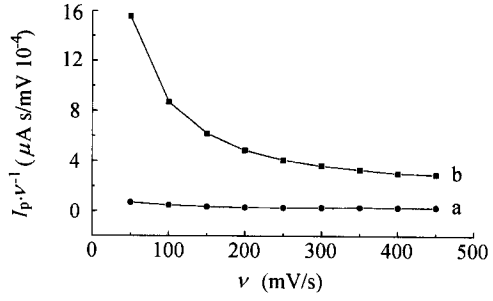
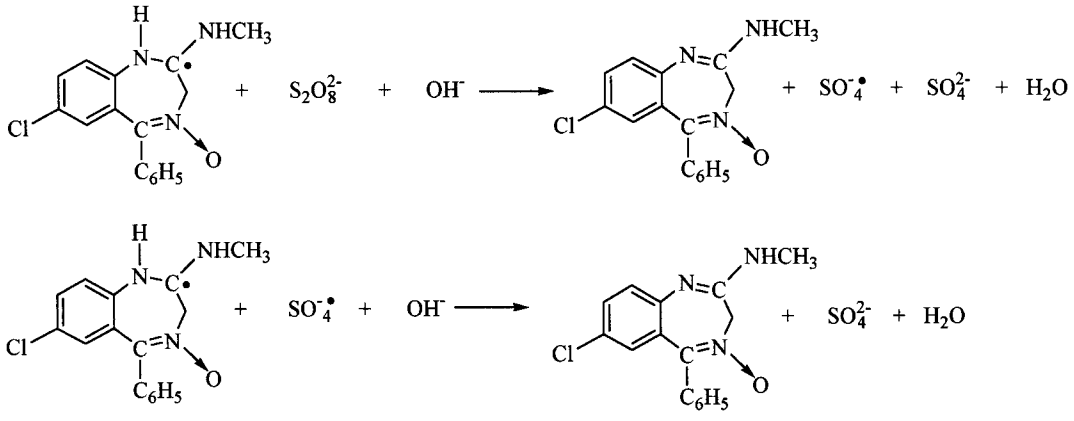


Fig. 3 Current function $I_p v^{-1}$ of chlordiazepoxide (8.0×10^{-6} mol/L) in (a) NH_3/NH_4Cl (pH 10.2 ± 0.1, 0.12 mol/L) solution and (b) NH_3/NH_4Cl (pH 10.2 ± 0.1, 0.12 mol/L) + $K_2S_2O_8$ (0.016 mol/L) solution.

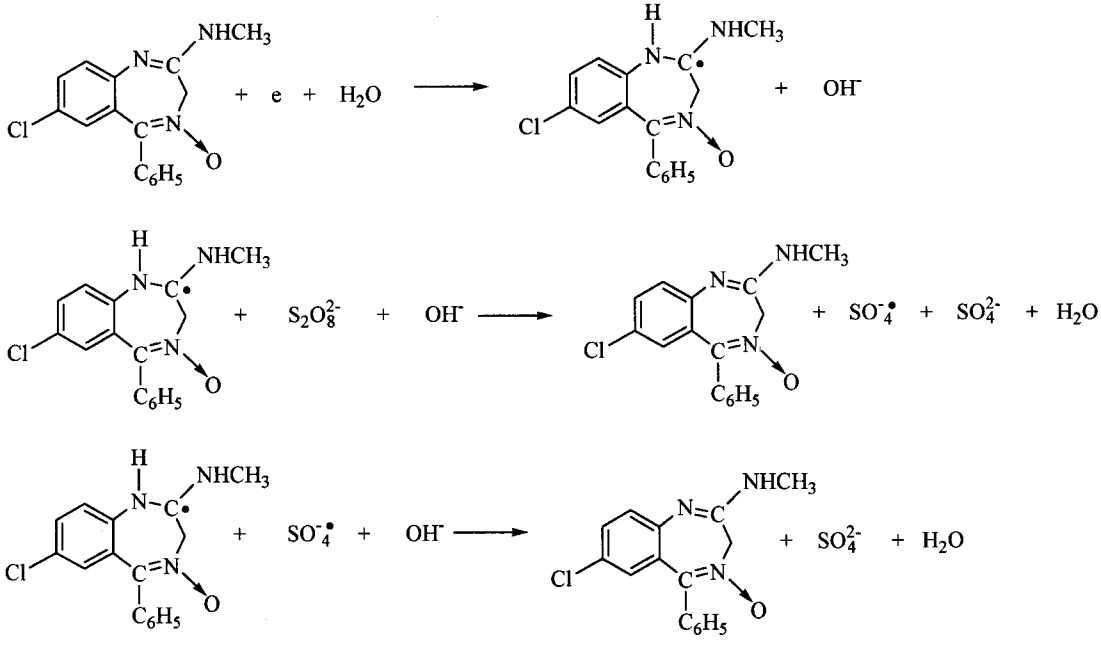
In summary, the mechanism of the catalytic wave of chlordiazepoxide was suggested as shown in scheme 3.

The production mechanism of the catalytic wave was again confirmed by cyclic voltammetric experiment in DMF/H_2O mixed solution. When adding $K_2S_2O_8$ into DMF/H_2O mixed solution, cyclic voltammogram (Fig. 4, curve b) showed that the wave P_1 increased sharply and the wave P_2 disappeared, whereas, a small reduction wave P_a appeared on reserve scan with the peak potential −1.32 V.

Scheme 2



Scheme 3



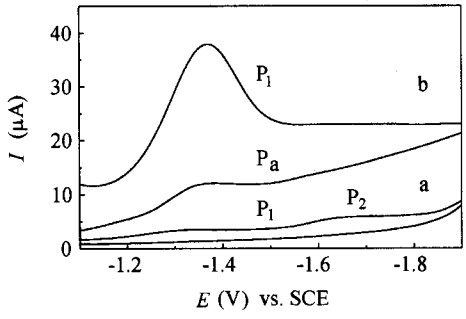


Fig. 4 Cyclic voltammograms of chlordiazepoxide (8.0×10^{-5} mol/L) in (a) DMF/$(C_2H_5)_4NBr$ (0.1 mol/L) solution and (b) DMF-$(C_2H_5)_4NBr$ (0.1 mol/L) + $K_2S_2O_8$ (0.010 mol/L) solution. Reverse scan from −1.90 V, scan rate 0.25 V/s.

Apparent rate constant of catalytic reaction

Under the conditions chosen in this work, the concentration of $K_2S_2O_8$ was much higher than that of chlordiazepoxide, so the oxidation reaction of the free radical of chlordiazepoxide by both the $S_2O_8^{2-}$ and the $SO_4^{-\cdot}$ can be treated as pseudo first-order one. According to the current equation of adsorption catalytic wave in the linear-potential scan polarography, when the potentials are highly negative with respect to the half-wave potential, the ratio of the catalytic current I_{pk} to the corresponding reduction current I_{pc} is described to the following relationship.[17]

$$I_{pk}/I_{pc} = 0.815(2k_f c_Z t)^{1/2}$$

where c_Z is $K_2S_2O_8$ concentration, the unit of $K_2S_2O_8$ concentration is mol/L; t is the sampling time, it is 6.4 s; The factor 2 indicates that one mole of $K_2S_2O_8$ oxidized two moles of the free radical intermediate of chlordiazepoxide; The linear potential scan rate v was 0.25 V/s. When $K_2S_2O_8$ concentration was in range of 4.0×10^{-3}—2.0×10^{-2} mol/L, the obtained relationship between the I_{pk}/I_{pc} and the $c^{1/2}_{K_2S_2O_8}$ was:

$$I_{pk}/I_{pc} = 166c^{1/2}_{K_2S_2O_8} (r = 0.9948,\ n = 6)$$

It was calculated that the apparent rate constant k_f of the oxidation reaction is 3.2×10^3 $mol^{-1} \cdot L \cdot s^{-1}$.

Application

Choice of experimental conditions

The nature, pH and concentration of the supporting electrolyte could, if not all, influence the voltammetric response. In NH_3/NH_4Cl buffer solution in the presence of $K_2S_2O_8$, the effects of pH and concentration of the supporting electrolyte on the peak current were examined. Experimental results indicated that a NH_3/NH_4Cl (pH 10.2 ± 0.1, 0.12 mol/L) buffer solution was the best because it resulted in higher and steadier peak current. Moreover, the peak current of chlordiazepoxide increased gradually with $K_2S_2O_8$ concentration increasing from 0.004 to 0.02 mol/L. When $K_2S_2O_8$ concentration was in the range of 0.016 mol/L—0.02 mol/L, the peak current achieved the maximum value. When $K_2S_2O_8$ concentration exceeded 0.02 mol/L, the peak current decreased slightly. Therefore, taking into account the peak shape and sensitivity, the optimal supporting electrolyte was 0.12 mol/L NH_3/NH_4Cl (pH 10.2 ± 0.1)/0.016 mol/L $K_2S_2O_8$ buffer solution.

Calibration curve

A second order derivative technique was used for measurement of peak current of the catalytic wave in the practical analysis because it has higher resolution capacity and can well eliminate background current in the presence of $K_2S_2O_8$. The profile of the catalytic wave of chlordiazepoxide was well defined, and the peak current was easy to be measured accurately. In the optimal supporting electrolyte, the second order derivative peak current was rectilinear to chlordiazepoxide concentration in the range of 3.20×10^{-8}—1.60×10^{-7}, 1.60×10^{-7}—1.44×10^{-6} and 1.44×10^{-6}—1.44×10^{-5} mol/L, respectively (Table 1). The limit of detection was 9.0×10^{-9} mol/L. The catalytic wave improved by 21 times in sensitivity as compared with the corresponding reduction wave for 8.0×10^{-6} mol/L chlordiazepoxide.

Sample analysis

The proposed method was more simple and sensitive, and was evaluated by the determination of chlordiazepoxide content in pharmaceutical tablets.

Preparation of samples Fine powder of 4 tablets was weighed accurately and was dissolved with 10 mL of HCl (1.0 mol/L), then diluted to 500 mL with water. Insoluble residual was filtered off from the obtained solution by dry filter method.

Measurement process A certain amount of chlordiazepoxide standard working solution or the sample solution, 0.3 mL of NH_4Cl (1.0 mol/L) solution, 2.7 mL of NH_3 (1.0 mol/L) solution and 4.0 mL of $K_2S_2O_8$ (0.1 mol/L) solution were successively added into a 25 mL volumetric flask. It then was diluted to the mark with water. The prepared solution was transferred to polarographic cell. The linear-potential scan was cathodically performed from −0.90 to −1.40 V. The second order derivative peak current of the catalytic wave with peak potential of −1.25 V was recorded. The calibration graph was cons-

Table 1 Calibration curve for chlordiazepoxide

Concentration range ($mol \cdot L^{-1}$)	Linear regression equation			
	Intercept ($\mu A \cdot s^{-2}$)	Slope [$\mu A \cdot s^{-2}/(mol \cdot L^{-1})$]	Correlation coefficient r	n
3.20×10^{-8}—1.60×10^{-7}	0.151	2.31×10^6	0.9989	8
1.60×10^{-7}—1.44×10^{-6}	0.417	7.26×10^5	0.9997	8
1.44×10^{-6}—1.44×10^{-5}	−0.012	1.01×10^6	0.9993	8

tructed by plotting the second order derivative peak current I_p'' of the catalytic wave against chlordiazepoxide concentration. The content of chlordiazepoxide was calculated according to the calibration graph. The results were given in Table 2. In addition, recovery test was carried out by addition of a certain amount of chlordiazepoxide standard working solution to different sample solution. The results were given in Table 3.

For the sake of evaluation of the proposed method, the same sample was determinated by HPLC method too. Under such chromatographic conditions as: methanol/water ($V/V = 60/40$) mobile phase and flow rate 0.8 mL/min, detection wavelength 309 nm, the experiments showed that the retention time of chlordiazepoxide was 8.575 min. The calibration plot obtained was $Y = -21.3 + 3.11 \times 10^3 c$ (mol/L) ($r = 0.99996$, $n = 5$). The results were given in Table 2. The results obtained by using the proposed method were in good agreement with those by using HPLC method.

Table 2 Determination results for chlordiazepoxide in tablets

This method		
Chlordiazepoxide found (mg/tablet)	Average (mg/tablet)	RSD (%)
9.65, 9.82, 9.50, 9.76, 9.47	9.64	1.6
HPLC method		
Chlordiazepoxide found (mg/tablet)	Average (mg/tablet)	RSD (%)
9.40, 9.43, 9.48, 9.42, 9.45	9.44	0.32

Table 3 Recovery results in sample

Sample/[10^{-6}/(mol·L^{-1})]	Added/[10^{-6}/(mol·L^{-1})]	Found/[10^{-6}/(mol·L^{-1})]	Recovery/(%)
5.14	1.12	6.27	100.9
5.14	1.28	6.44	101.56
5.14	1.44	6.57	99.31
10.28	1.12	11.38	98.21
10.28	1.28	11.52	96.88
10.28	1.44	11.70	98.61

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