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Short communication

Polarographic determination of diazepam with its parallel catalytic wave in the presence of persulfate

Wei Guo [a,*], Hong Lin [b], Limin Liu [a], Junfeng Song [a]

[a] *Department of Chemistry, Northwest University, Xi'an 710069, China*
[b] *Department of Chemistry, Yuxi teachers College, Yuxi 653100, China*

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Abstract

A new method for the determination of diazepam was proposed based on its polarographic catalytic wave in the presence of persulfate. In 0.20 M NaAc–HAc (pH 4.7)–2.0×10^{-2} M $K_2S_2O_8$ supporting electrolyte, the reduction wave of diazepam with peak potential −0.89 V (versus SCE) was catalyzed, producing a parallel catalytic wave. The peak current of the catalytic wave was 15 times higher than that of the corresponding reduction wave for 4.0×10^{-6} M diazepam, and was rectilinear to diazepam concentration in the range of 5.6×10^{-8} to 8.8×10^{-6} and 8.8×10^{-6} to 2.0×10^{-4} M. The detection limit was 9.6×10^{-9} M. The mechanism of the parallel catalytic wave of diazepam was discussed.

Keywords: Diazepam; Persulfate; Polarographic catalytic wave

1. Introduction

Diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H, 1,4-benzodiazepam-2-one) has been widely used as tranquilizers for the treatment of diseases of the central nervous system. Consequently, the need arose for the determination of diazepam in tablet, blood, urine, etc. Liquid chromatography and non-aqueous titrimetry were recommended by the Pharmacopeia [1,2]. Derivative UV spectroscopy [3,4], HPLC [5–8], potentiometric [9], polarographic [10–12] and voltammetric [13] methods had been reported. Among these methods, the differential plus polarographic and adsorptive stripping voltammetric methods based on the reduction wave of diazepam were of higher analytical sensitivity. Ellaithy et al. [10], Sreedhar and Reddy [11] and Guadalupe Garcia et al. [12] applied differential pulse polarography to the determination of diazepam. The detection limits obtained were about 2.0×10^{-7} M in magnitude. Kalvoda [13] reported an adsorptive stripping voltammetric method. When diazepam was accumulated at potential of −0.50 V for 3 min in acetate buffer (pH 4.6), the detection limit achieved by differential pulse voltammetry was 1.0×10^{-9} M.

Besides differential pulse polarography and adsorptive stripping voltammetry, the polarographic method based on the so-called catalytic wave of organic compound in the presence of suitable oxidant is a simple and effective pathway to improve the analytical

* Corresponding author. Tel.: +86-29-8302604; fax: +86-29-8303448.
E-mail address: songjunf@nwu.edu.cn (W. Guo).

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sensitivity. In such polarographic catalytic wave, polarographic reduction of the reducible groups of organic compounds was simultaneous with chemical regeneration of the reducible groups through oxidation of their free radicals by oxidant on electrode surface. Recently, the polarographic catalytic waves of organic compounds such as sulfur-containing amino acid [14], protein [15–18], steroid [19], unsaturated carbonyl acid [20], flavonone [21], isoquinolinium alkaloid [22] and others in the presence of such oxidants as KIO_3, H_2O_2 or $K_2S_2O_8$ have been reported. Using the polarographic catalytic wave for the determination of these organic compounds, the improvement of analytical sensitivity was one or two orders of magnitude. To our knowledge, however, the polarographic catalytic wave of diazepam in the presence of oxidant has never been reported.

The aim of this work was to develop a new method for the determination of diazepam based on its parallel catalytic wave in the presence of $K_2S_2O_8$ and to discuss the mechanism of the catalytic wave of diazepam briefly.

2. Experimental

2.1. Reagents and chemicals

Diazepam is of biochemical-reagent grade and was purchased from Xi'an Pharmaceutical Plant (Xi'an, China). 1.0×10^{-2} M stock solutions of diazepam in N, N-dimethylformamide (DMF) and water were prepared, respectively. Other standard working solutions of diazepam were obtained by diluting the stock solution with DMF and water, respectively. Diazepam tablets (labeled amount 5 mg per tablet) were purchased from Northwest University Hospital (Xi'an, China). Anhydrous DMF is of analytical-reagent grade and was purchased from Xi'an Chemical Reagent Factory (Xi'an, China). Other chemicals are of analytical-reagent grade. Twice distilled water was used throughout the experiments.

2.2. Apparatus

The determination of diazepam was performed by model JP-2 linear-potential scan polarograph (Chengdu Instrumental Factory, China). The polarograph was equipped with a three-electrode system consisting a dropping mercury working electrode (DME), a platinum wire counter electrode and a saturated calomel reference electrode (SCE). The automatically controlled drop-time of DME is 7 s, and a potential scan rate v is $0.25\,V\,s^{-1}$. The polarogramms were recorded by second-order derivative set of the linear-potential scan polarograph. Cyclic voltammetric experiments were carried out on model CH660 electrochemical workstation (CH Instrument, USA) that was controlled by CH660 software and worked under Windows 98 environment. The workstation was contacted with Model 303A SMDE system (EG&G PARC, USA) involving a hanging mercury drop working electrode in middle size, a platinum wire counter electrode and a SCE reference electrode. The potential scan rate v is $0.25\,V\,s^{-1}$. An Orion pH-meter (Model 237) was used for the pH measurements.

Spectrophotometric determination of diazepam was carried out by AIC UV-900 spectrophotometer (Beijing Ruili Instrument, China). A cell of 1-cm path length and a wavelength of 284 nm were used for measuring the absorbances of diazepam against the 0.5% H_2SO_4–methanol blank solution.

2.3. Determination procedure of sample

Fine powder of 10 tablets was weighed accurately and solved with water in 50 ml standard volumetric flask, then diluted by 50 times with water again.

Suitable amounts of supernatant liquid of sample or standard working solutions of diazepam, 5.0 ml of 1.0 M NaAc–1.0 M HAc buffer solution and 5.0 ml of 0.1 M $K_2S_2O_8$ solution were successively added into a 25 ml standard volumetric flask, and was diluted to the mark with water. The prepared solution was transferred into a polarographic cell without deaeration, the linear-potential scan was performed cathodically from −0.60 to −1.10 V on Model JP-2 linear-potential scan polarograph. The second-order derivative polarogram was recorded and the second-order derivative peak current of the catalytic wave was measured. The experiments were carried out at room temperature. The calibration curve was obtained by least-squares linear regression of the second-order derivative peak current of the catalytic wave versus the

concentrations of diazepam standard. Diazepam contents in tablets were calculated from the obtained calibration curve.

3. Results

3.1. Choice of medium and oxidant

To optimize the medium condition for polarographic catalytic wave of diazepam, both the type of medium and oxidant and pH value of medium used were chosen. Strongly acidic media (pH < 3) should be not used because diazepam is susceptible to acid hydrolysis with subsequent ring opening [12]. First, slightly acidic and alkaline media such as HAc–NaAc (pH 3.8–5.6), KH_2PO_4–Na_2HPO_4 (pH 5.5–8.0) and $NH_3{\cdot}H_2O$–NH_4Cl (pH 8.0–10.7) buffers were used to examine the reduction wave of diazepam itself. Experimental results were in agreement with that of the previous works by Ellaithy et al. [10], Guadalupe Garcia et al. [12] and Kalvoda [13]. The slightly acidic buffer, HAc–NaAc buffer, was chosen in which the reduction wave of diazepam itself was more sensitive than that in alkaline media. Second, the catalytic action of oxidants such as KIO_3, H_2O_2 and $K_2S_2O_8$ on the reduction wave of diazepam in the acetate buffer was examined. All these oxidants tested can cause a polarographic catalytic wave of diazepam. However, the catalytic action of $K_2S_2O_8$ was the most high in three oxidants tested. Therefore, a HAc–NaAc buffer containing $K_2S_2O_8$ was chosen as supporting electrolyte.

Polarographical reduction of electroactive $S_2O_8^{2-}$ had started at far positive potential than that of diazepam under the condition of this work [23]. When $S_2O_8^{2-}$ concentration was fixed, the reduction of $S_2O_8^{2-}$ produced a relatively high and constant background current in the potential domain to record the polarogram. The background current often made the current detector of the polarograph overflow when high sensitivity gear of the current detector was used, and brought a difficult for measuring the catalytic wave of trace of diazepam. Thus, second-order derivative technique was used to record the polarogram in this work as derivative technique can effectively eliminate the background current. As shown in Fig. 1, the second-order derivative profile

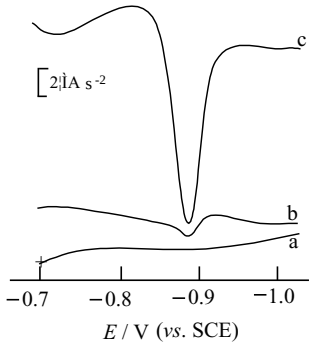


Fig. 1. Second-order derivative linear potential scan polarogram of diazepam in (a) 0.20 M NaAc–HAc (pH 4.7) buffer; (b) a + 4.0×10^{-6} M diazepam; (c) b + 4.0×10^{-3} M $K_2S_2O_8$. Initial potential −0.60 V, scan rate 0.25 V s^{-1}.

of the catalytic wave of diazepam was well defined, and the peak current can be measured accurately too.

3.2. Effect of pH value and buffer concentration

In HAc–NaAc buffer containing 2.0×10^{-2} M $K_2S_2O_8$, the effects of pH value and total concentration of the acetate buffer on the second-order derivative peak current i''_p and the peak potential E_p of the catalytic wave were examined. With pH value increasing from 3.8 to 5.3, the E_p shifted to negative direction. Meanwhile, the i''_p increased with pH value increasing from 3.8 to 4.5. Over the pH range of 4.5–4.9, the i''_p reached a maximum value and remained unchanged. When pH value was above 4.9, the i''_p decreased with pH value increasing. Thus the pH 4.7 of the acetate buffer was used. At pH 4.7, the peak potential of the catalytic wave was −0.89 V.

In addition, when the total concentration of the acetate buffer with the fixed pH 4.7 increased from 8.0×10^{-2} to 3.2×10^{-1} M, the E_p remained nearly unchanged. Moreover, the i''_p increased with the total concentration increasing from 8.0×10^{-2} to 2.0×10^{-1} M. When the total concentration was 2.0×10^{-1} M, the i''_p achieved the maximum value. While it exceeded 2.0×10^{-1} M, the i''_p decreased slightly. Thus, the total concentration of the acetate buffer (pH 4.7) used was 0.2 M that consists of 0.1 M HAc and 0.1 M NaAc solutions.

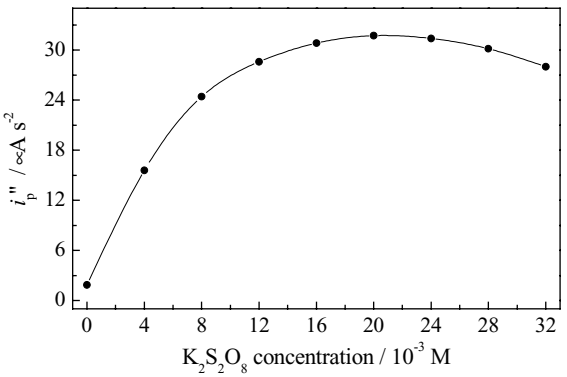


Fig. 2. Effect of $K_2S_2O_8$ concentration on peak current of 1.0×10^{-6} M diazepam in 0.20 M NaAc–HAc (pH 4.7) buffer.

3.3. Effect of $K_2S_2O_8$ concentration

The dependence of the second-order derivative peak current i_p'' of diazepam on $K_2S_2O_8$ concentration was shown in Fig. 2. When $K_2S_2O_8$ was absent, the i_p'' of the reduction wave was small. When $K_2S_2O_8$ was present, the E_p of the reduction wave of diazepam remained unchanged and the i_p'' increased greatly. And the i_p'' increased gradually with $K_2S_2O_8$ concentration increasing from 4.0×10^{-3} to 2.4×10^{-2} M. While $K_2S_2O_8$ concentration was in the range of 1.6×10^{-2} to 2.4×10^{-2} M, the i_p'' achieved the maximum value, and was 15-fold higher than that in the absence of $K_2S_2O_8$. The i_p'' decreased slightly when $K_2S_2O_8$ concentration exceeded 2.4×10^{-2} M. Thus, $K_2S_2O_8$ concentration chosen was 2.0×10^{-2} M.

Therefore, the optimal supporting electrolyte was a 0.2 M HAc–NaAc buffer (pH 4.7) containing 2.0×10^{-2} M $K_2S_2O_8$ for the determination of diazepam.

3.4. Validation of the proposed method

Calibration curves obtained were linear over diazepam concentration range of 5.6×10^{-8} to 8.8×10^{-6} and 8.8×10^{-6} to 2.0×10^{-4} M, respectively. Good correlation and slope reproducibility were observed. The linear regression equations obtained on five different days were i_p'' (μA s^{-2}) $= 0.5 + 7.0 \times 10^6$ c (M), ($n = 8$, $r = 0.9985 \pm 0.0011$) in the range of 5.6×10^{-8} to 8.8×10^{-6} M and i_p'' (μA s^{-2}) $= -0.4 + 7.1 \times 10^6$ c (M), ($n = 8$, $r = 0.9991 \pm 0.0004$) in the range of 8.8×10^{-6} to 2.0×10^{-4} M. The coefficients of variation of the slopes and the intercepts of the calibration curves were <2.1 and 1.5%, respectively. The detection limit ($S/N = 3$) was 9.6×10^{-9} M.

After adding the standard working solution and sample solution of diazepam into the optimal supporting electrolyte, the second-order derivative peak currents i_p'' of diazepam were continually measured. The currents remained nearly unchanged for at least 4 h.

The standard containing 1.0×10^{-7} and 8.0×10^{-7} M diazepam was analyzed by seven independent measurements during one day and on four consecutive days. The relative standard deviation of the i_p'' of diazepam was 1.2 and 0.9 % for repeatability and 1.8 and 1.6% for reproducibility, respectively.

Experiments showed that the excipients (starch, talcum, etc.) in tablet did not interfere with the determination. Diazepam tablets in the same batch (labeled amount 5 mg per tablet) were assayed by replicate determinations ($n = 6$) during 1 day. The average content of diazepam obtained by using the proposed method was 4.83 mg per tablet with relative standard deviation 1.2%. The average content obtained by using the spectrophotometric method of the Chinese Pharmacopoeia [24] was 4.84 mg per tablet with relative standard deviation 1.1%. As can be seen, there were no significant differences between the proposed method and the spectrophotometric method. The tablet was repeatedly analyzed by the proposed method on three different days. The relative standard deviation of the diazepam contents was 1.7%. The results indicated that the proposed method has acceptable interday and intraday precision and accuracy. Furthermore, recovery was tested with adding three different amounts of diazepam standard working

Table 1
Recovery results in sample

Sample (10^{-6} M)	Added (10^{-6} M)	Found (10^{-6} M)	Recovery (%)	RSD (%)[a]
3.41	2.4	5.73	96.7	
3.41	4.0	7.47	101.5	
3.41	5.6	8.87	97.5	1.93
1.70	2.4	4.07	98.8	
1.70	4.0	5.62	98.0	
1.70	5.6	7.35	100.9	

[a] Relative standard deviation (RSD) for six recovery tests.

solution to sample solutions at two levels, respectively. Each recovery was calculated by comparing the results obtained before and after the addition. The results were shown in Table 1. The recoveries were between 96.7 and 101.5%. The average recovery for six tests was 98.9% with relative standard derivation 1.93%.

4. Discussions

4.1. Characterization of the catalytic wave

The fact that the presence of $K_2S_2O_8$ in 0.20 M NaAc–HAc buffer (pH 4.7) made the peak current of the reduction wave of diazepam increase greatly and the peak potential unchanged (Fig. 3, curve b) indicated that the enhanced wave was a polarographic catalytic one. The kind of polarographic wave can be diagnosed by the dependence of the current function $i_p \cdot v^{-1/2}$ on potential scan rate v. The dependences of the current function $i_p \cdot v^{-1/2}$ of the corresponding reduction wave and of the catalytic wave on potential scan rate v showed in Fig. 4. The current function $i_p \cdot v^{-1/2}$ of the catalytic wave (Fig. 4, curve b) was different from that of the reduction wave (Fig. 4, curve a). The current function $i_p \cdot v^{-1/2}$ of the catalytic wave at first decreased with potential scan rate v increasing in the range of 0.1–2.0 V s^{-1}, then leveled off with scan rate v further raising. Moreover, the peak current i_{p1} of the catalytic wave increased with $K_2S_2O_8$ concentration increasing from 4.0×10^{-3} to 2.4×10^{-2} M, and the ratio of the i_{p1} to the peak current i_{pc} of the corresponding reduction wave was linearly proportional to the square root of $K_2S_2O_8$ concentration in range of 4.0×10^{-3} to 2.4×10^{-2} M. The linear regression equation obtained was $i_{p1}/i_{pc} = 106.1\ c^{1/2}_{K_2S_2O_8}$ ($n = 6$, $r = 0.9957$). These characters demonstrated that the catalytic wave of diazepam in the presence of $K_2S_2O_8$ was a parallel one [25].

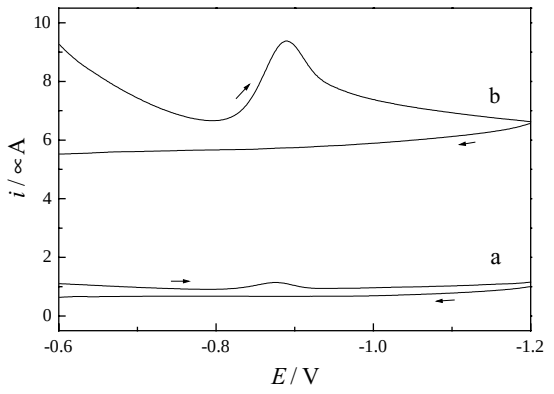


Fig. 3. Cyclic voltammograms of 1.0×10^{-6} M diazepam in (a) 0.20 M NaAc–HAc (pH 4.7) buffer; (b) a + 0.02 M $K_2S_2O_8$ solution. Scan rate 0.25 V s^{-1}.

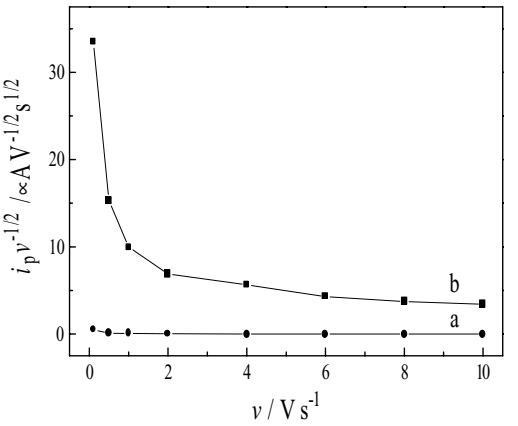


Fig. 4. Current function $i_p \cdot v^{-1/2}$ of 8.0×10^{-6} M diazepam in (a) 0.20 M NaAc–HAc (pH 4.7) buffer; (b) a + 0.02 M $K_2S_2O_8$ solution.

4.2. Mechanism of the catalytic wave

The polarographic behavior of diazepam and its analogues such as tetrazepam, nontetrazepam and menitrazepam has been studied previously [10,12,13]. The two-electron and two-proton reduction wave of diazepam over the whole pH-range was attributed to reduction of the 4,5 N=C double bonds via free radical intermediate. However, no evidences of both the presence of the free radical and subsequent reaction of the electrogenerated free radical were given. Generally speaking, proton conditions of media largely influenced the successive electron transfer process via free radical of organic compound. Therefore, voltammetric behaviors of diazepam in aprotic medium, DMF–$(C_2H_5)_4NBr$ medium, with a hanging mercury drop electrode was examined in order to illustrate the mechanism of the catalytic wave of diazepam in the presence of $K_2S_2O_8$.In the DMF–0.1 M $(C_2H_5)_4NBr$

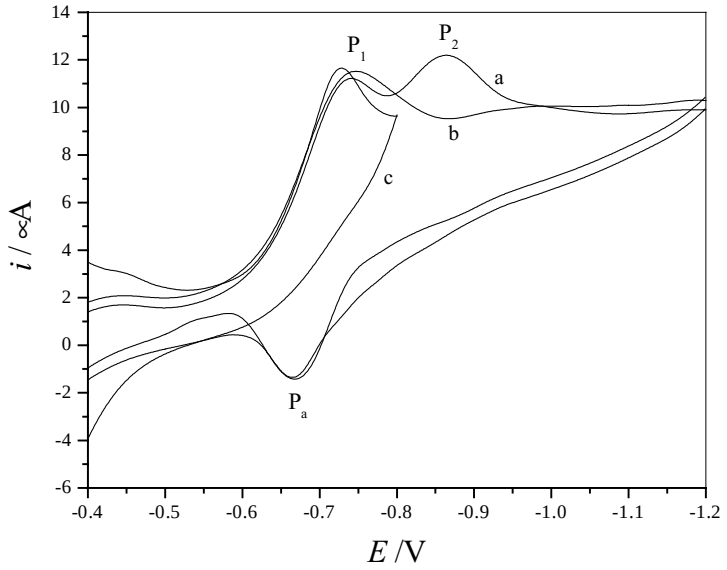
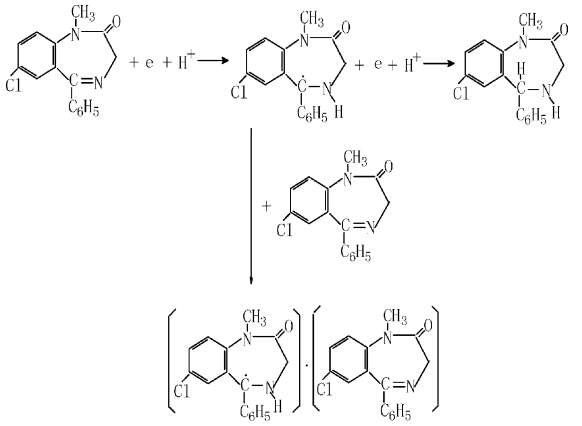


Fig. 5. Cyclic voltammograms of 8.0×10^{-6} M diazepam in DMF–0.1 M $(C_2H_5)_4NBr$ medium. (a) initial potential −0.40 V, potential of reversal −1.20 V, remained time at −0.80 V is 0 s; (b) initial potential −0.40 V, potential of reversal −1.20 V, remained time at −0.80 V is 10 s; (c) initial potential −0.40 V, potential of reversal −0.80 V; scan rate 0.25 V s^{-1}.

medium containing 8.0×10^{-6} M diazepam, cyclic voltammogram was showed in Fig. 5, curve a. There were two reduction waves, P_1 and P_2, of diazepam on cathodic scan from −0.40 to −1.20 V and an oxidation wave P_a on reverse scan, respectively. The peak potential E_{P1} of the wave P_1 was located at −0.73 V, the E_{P2} of the wave P_2 at −0.86 V, and the E_{pa} of the wave P_a at −0.67 V. The peak current i_{p1} of the wave P_1 was higher than the peak current i_{p2} of the wave P_2. With potential scan rate v increasing from 0.1 to 5.0 V s^{-1}, the peak currents, the i_{p1} and i_{p2}, of both the wave P_1 and the wave P_2 increased to different degree, in the meanwhile, the peak current i_{pa} of the wave P_a decreased. The peak potential E_p of three waves shifted negatively with the v increasing. For wave P_1, the linear equation of the E_{p1}–log v relationship was $-E_{p1} = 0.72 + 0.0298 \log v$ ($n = 5$, $r = 0.9986$). In addition, when the cyclic voltammetric experiment was performed in the potential domain of −0.40 to −0.80 V, no oxidation wave P_a appeared on reverse scan except the wave P_1 on cathodic scan (Fig. 5, curve c). This indicated that the wave P_a did not correspond to the wave P_1. To further verify the nature of the wave P_a, cyclic voltammetry in the potential domain of −0.40 to −1.20 V was performed in such operation as the potential kept first at −0.80 V for several seconds after the potential scanned cathodically from −0.40 to −0.80 V, and then subsequently scanned from −0.80 to −1.20 V and from −1.20 to −0.40 V followed on. The experimental results showed that, the longer the remained time of the potential at −0.80 V before subsequent scans was, the lower the i_{p2} was and the higher the i_{pa} was. When the remained time was 10 s, the wave P_2 disappeared and the i_{pa} of the wave P_a increased (Fig. 5, curve b). These facts described above demonstrated that the N=C bond in 4,5 position of diazepam was firstly reduced to an intermediate free radical, producing the wave P_1. The free radical was further reduced, producing the wave P_2 along with a subsequent reaction. From the slope value 0.0298 of the E_{p1}–log v relationship of the wave P_1, it can be deduced that a father–son reaction between the generated free radical and a neutral molecule of diazepam happen [26–29], and the wave P_a was oxidation wave of the product of the father–son reaction. Obviously, these two reduction waves in DMF media corresponded to the single reduction wave in aqueous solution.

So the reduction process of diazepam was suggested as follows:



Evidently, the production of the parallel catalytic wave of diazepam was ascribed to that the electrogenerated free radical of diazepam reduction was oxidized by oxidant $S_2O_8^{2-}$. However, the oxidation reaction may involve two successive steps as the reduction of $S_2O_8^{2-}$, regardless of polarographically and chemically, was two successive one-electron process via intermediate sulfate radical $SO_4^{-\bullet}$ [23,30]. From standard reduction potential of $S_2O_8^{2-}$ in aqueous solution

$$S_2O_8^{2-} + 2e \rightarrow 2SO_4^{2-} \qquad E^{\phi} = 2.0\,V$$

$$S_2O_8^{2-} + e \rightarrow SO_4^{-\bullet} + SO_4^{2-} \qquad E^{\phi} \leq 0.6\,V$$

$$SO_4^{-\bullet} + e \rightarrow SO_4^{2-} \qquad E^{\phi} \geq 3.4\,V$$

$SO_4^{-\bullet}$ is stronger than $S_2O_8^{2-}$ in oxidisability. Therefore, it is sure that $SO_4^{-\bullet}$ participated in the oxidation reaction as well. The $SO_4^{-\bullet}$ resulted from both chemical and electrochemical reductions of $S_2O_8^{2-}$. On the one hand, when $S_2O_8^{2-}$ met the free radical of diazepam, it chemically oxidized the free radical and $S_2O_8^{2-}$ itself was first reduced to $SO_4^{-\bullet}$. The generated $SO_4^{-\bullet}$ immediately oxidized other free radical of diazepam in very fast velocity because the reaction of $SO_4^{-\bullet}$ with the free radical of diazepam was one between both free radicals. On the other hand, all the polarographic and voltammetric experiments in this work were carried out in such potential domain as it was negative enough for $S_2O_8^{2-}$ to be reduced via the $SO_4^{-\bullet}$ on electrode surface too. There was the $SO_4^{-\bullet}$ from electrochemical reduction of $S_2O_8^{2-}$ on electrode surface. Therefore, both $S_2O_8^{2-}$ and $SO_4^{-\bullet}$ together oxidized the intermediate free radical of diazepam to regenerate the original N=C bond, producing the parallel catalytic wave of diazepam.

In addition, the mechanism of the catalytic wave was reconfirmed by experiment in DMF–$(C_2H_5)_4NBr$ media. When adding $K_2S_2O_8$ into DMF medium containing diazepam, cyclic voltammogram (Fig. 6) showed that the reduction wave P_1 increased sharply,

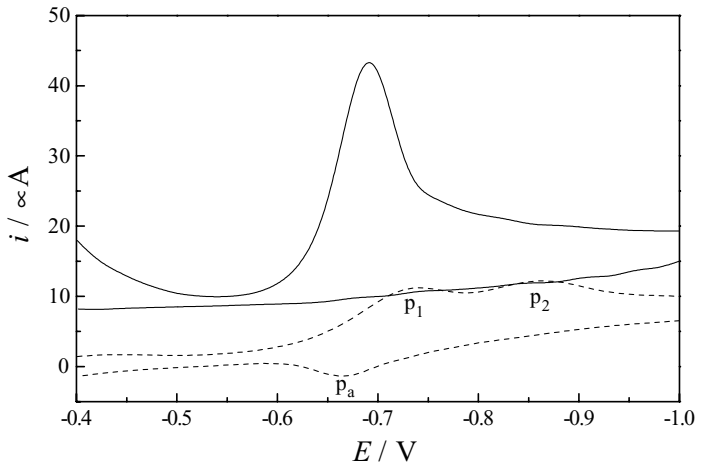
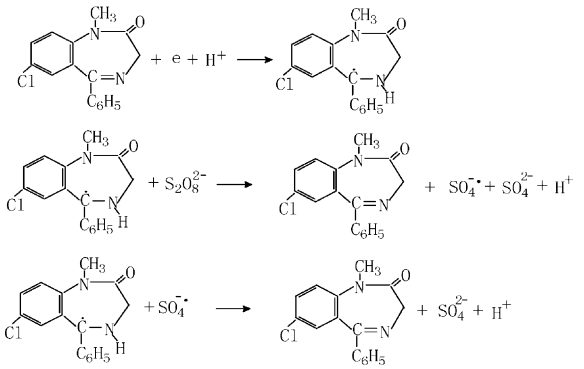


Fig. 6. Cyclic voltammogram of 8.0×10^{-6} M diazepam in DMF–0.1 M $(C_2H_5N)_5Br$ medium in the absence (dashed line ---) and in the presence (solid line —) of 0.01 M $K_2S_2O_8$ solution. Initial potential −0.40 V, potential of reversal −1.00 V and scan rate 0.25 V s^{-1}.

both the reduction wave P_2 and the oxidation wave P_a disappeared. In summary, the production scheme of the catalytic wave of diazepam was suggested as follows:

$$S_2O_8^{2-} + e \rightarrow SO_4^{-\bullet} + SO_4^{2-}$$



5. Conclusion

The polarographic catalytic wave of diazepam was easily obtained by both adding oxidant persulfate in the supporting electrolyte and using conventional polarograph, such as linear-potential scan polarograph. The polarographic catalytic wave method for the determination of diazepam has the advantage of the differential plus polarographic and adsorptive stripping voltammetric methods in simplicity in operation. Analytical sensitivity had been improved to certain degree. If a previously adsorptive concentration procedure were coupled with the catalytic wave method, higher analytical sensitivity would be achieved.

Acknowledgements

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