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Voltammetric Determination of Oxazepam and Lorazepam

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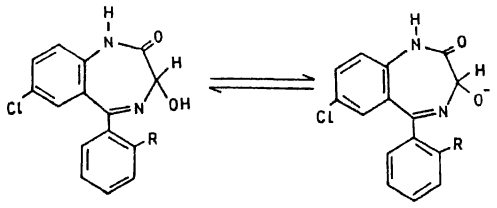
Voltammetric reduction behaviour of oxazepam and lorazepam (sedative-hypnotic drugs) has been studied by employing d.c. polarography, cyclic voltammetry, a.c. polarography, differential pulse polarography and rotating ring disk voltammetry in the universal buffers of pH ranging from 2.0 to 12.0. These compounds show a single well defined wave/peak in the entire buffer systems studied. This single wave/peak is attributed to the simultaneous reduction of the 4,5-azomethine bond and reductive cleavage of the hydroxyl group in the 3-position of the 1,4-diazepine ring, corresponding to two electrons each. Differential pulse polarographic method has been developed for the quantitative determination of title compounds in different pharmaceutical formulations. Kinetic parameters are evaluated and a reduction mechanism is proposed based on the results obtained.

Since the introduction of chlordiazepoxide hydrochlorides in 1960[1], a large number of 1,4-benzodiazepine compounds have been investigated as tranquillisers, hypnotics, sedatives and antidepressants[2].

Oxazepam,7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one (**1**) and lorazepam, 7-chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one (**2**) have a more pronounced hypnotic action than other benzodiazepines. Several methods have been described for the determination of benzodiazepines based on electron-capture gas liquid chromatagraphy[3], luminescence determination on thin layer chromatographic plates[4] and high-pressure liquid chromatography[5]. The present paper describes a new polarographic method for the determination of the above two compounds in different pharmaceutical formulations; time-consuming separation of excipient prior to the determination is not necessary in this method.



1 ; R = H
2 : R = Cl

Results and Discussion

Oxazepam and lorazepam show a single well-defined wave/peak in the entire buffer system studied over the pH range 2.0–12.0. This peak is attributed to the simultaneous reduction of the 4,5-azomethine bond and reductive cleavage of the hydroxyl group in the 3-position of the 1,4-diazepine ring[6], corresponding to two-electrons each. However, in alkaline media (pH > 8.0), oxazepam and lorazepam exhibit a distinct polarographic behaviour in which they give rise to relatively small broad peaks at substantially more negative potentials. This effect is a polarographic manifestation of the acid-base equilibrium in which the ionic species is reduced at a more negative potential than the neutral molecule and is also due to repulsion of the anion from the negatively charged mercury surface. Typical voltammograms are shown in Figs. 1–5.

The reduction process in both the compounds is found to be diffusion-controlled and adsorption free in all the buffer systems studied as evidenced from the linear plots of i_d vs $h^{1/2}$, i_d vs C, i_p vs $V^{1/2}$ and i_m vs $t^{2/3}$ which pass through the origin. The current function $i_p/CV^{1/2}$ is found to be fairly constant with respect to scan rate (V) indicating the electrode processes to be free from adsorption complications. The collection efficiencies (N) calculated from rotating ring disk voltammetry,

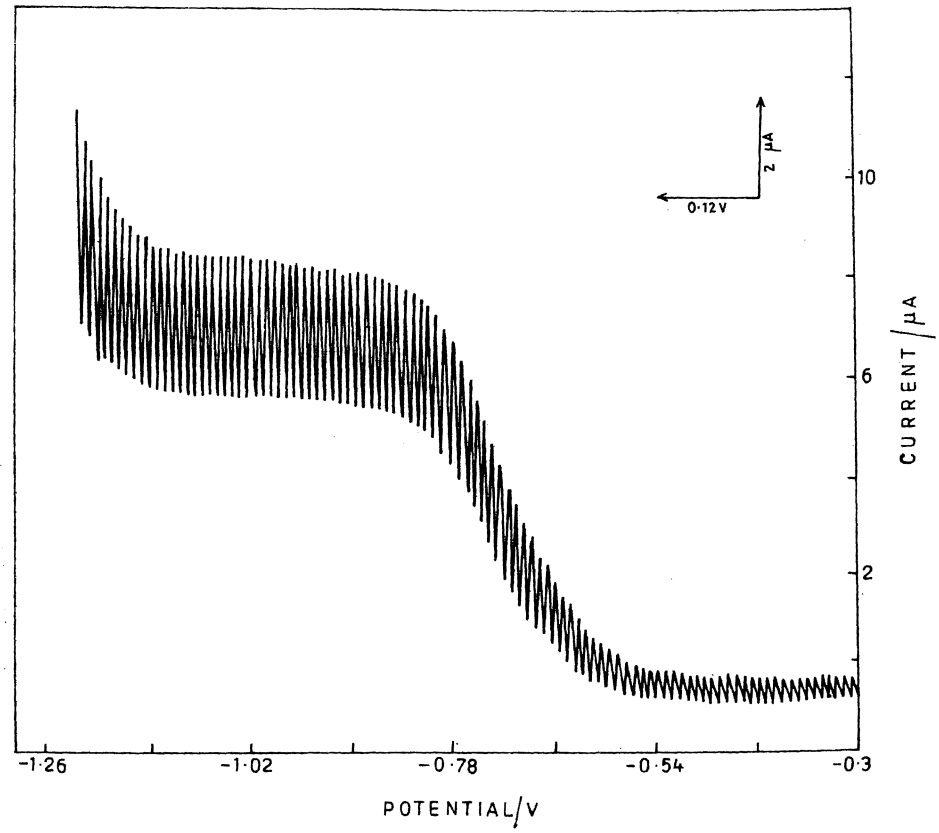


Fig. 1. Typical D.C. polarogram of oxazepam in pH = 2.0; conc.. = 0.5 m*M*, drop time = 3 s.

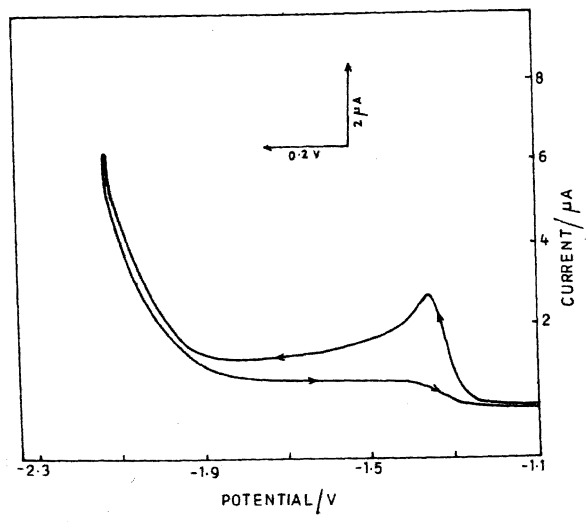


Fig. 2. Typical cyclic voltammogram of oxazepam in pH = 10.0; concn. = 0.5 m*M*, scan rate = 40 mV s^{-1}.

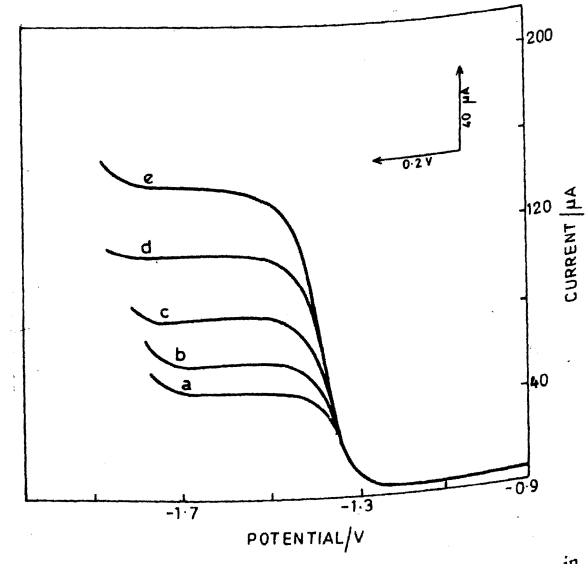


Fig. 3. Typical rotating disk voltammogram of oxazepam in pH = 10.0; concn. = 0.5 m*M*, sweep rate = 50 mVs^{-1}; (a) 500, (b) 700, (c) 1000, (d) 1500 and (e) 2000 rpm.

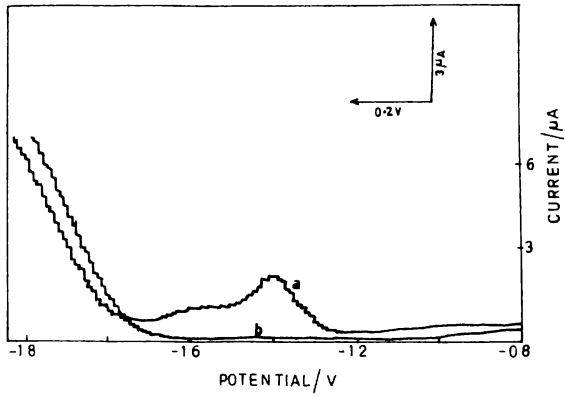


Fig. 4. Typical a.c. polarogram of larazepam in pH = 10.0; concn. = 0.5 m*M*, drop time = 3 s : (a) a.c. peak and (b) base line.

0.176 for oxazepam and 0.177 for lorazepam, evaluated in different pH zones, are seen to be in good agreement with the theoretical value (0.179), also indicating the absence of kinetic complications.

The irreversible nature of the two waves/peaks obtained for both the compounds are seen from log-plot analysis, disobedience of Tomes' criterion and non-linearity between i_m vs $(1-\sigma)/(1+\sigma)$ plots. In the above compounds, $E_{1/2}/E_p$ values are found to be dependent on pH, which shifted towards more negative potentials with the increase in pH of the buffer systems. An increase in the percentage of DMF in the polarographic test solution is seen to shift the half-wave potentials towards more negative value with simultaneous decrease in diffusion current, which may be explained as due to the possible absorption of the solvent molecules on the surface of the electrode and also due to the increase in viscosity of the medium.

Electrochemical reduction of azomethine group containing compounds follows, in general, a two-electron course leading to saturation of azomethine group. Millicoulometric and controlled potential electrolysis results of these compounds also confirm the same. Millicoulometric results showed the number of electrons to be four for oxazepam and lorazepam due to the simultaneous reduction of the azomethine and protonated hydroxyl groups in the different pH zones evaluated. Controlled potential electrolysis is carried out in pH 4.0 at –0.95 V vs SCE

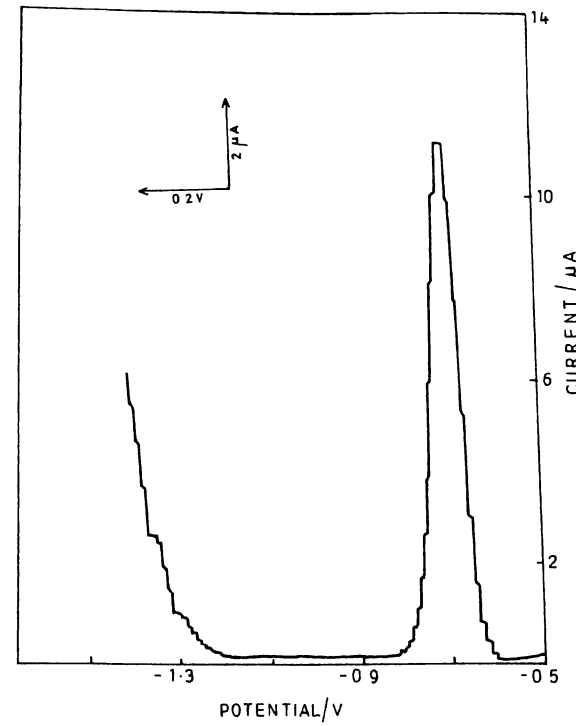


Fig. 5. Typical differential pulse polarogram of lorazepam in pH = 2.0; concn. = 0.5 m*M*, drop time = 2 s.

and the products are identified as the corresponding saturation products by ir spectral studies.

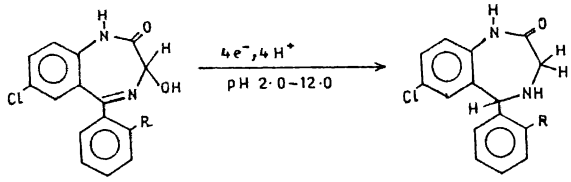
Kinetic parameters such as diffusion coefficient (D) and heterogeneous forward rate constant ($K^0_{f,h}$) values evaluated at various pH zones by different techniques are furnished in Table 1. The adsorption free nature of electrode process is clearly evidenced from the nearly equal diffusion coefficient values obtained from all the techniques. The rate constant values are found to decrease with increase in pH of the supporting electrolyte. The $K^0_{f,h}$ values are found to be very low in basic media due to the acid-base equilibrium and repulsion of the anion from the negatively charged mercury surface. Lorazepam is found to reduce more easily when compared to oxazepam due to the presence of electron-withdrawing chlorine on the 5-*o*-phenyl group. The same kind of results are observed from the comparison of the reduction potentials of the above two compounds. Hence the ease of reduction is observed to follow the order : lorazepam > oxazepam.

TABLE 1–TYPICAL ELECTROCHEMICAL DATA OF OXAZEPAM AND LORAZEPAM

Concn. = 0.5 m*M*

Supporting electrolyte of pH	D. C. polarography Drop time = 3 s			Cyclic voltammetry Scan rate = 40 mV s^{-1}			A. C. polarography Drop time = 3 s			Differential pulse polarography Drop time = 2 s			Rotating disk voltammetry Sweep rate = 50 mV s^{-1}	
	$-E_{1/2}$ V	$D\times10^5$ $cm^2 s^{-1}$	$k^0_{f,h}$ cm s^{-1}	$-E_p$ V	$D\times10^5$ $cm^2 s^{-1}$	$k^0_{f,h}$ cm s^{-1}	$-E_s$ V	$D\times10^5$ $cm^2 s^{-1}$	k_s cm s^{-1}	$-E_m$ V	$D\times10^5$ $cm^2 s^{-1}$	$k^0_{f,h}$ cm s^{-1}	$D\times10^5$ $cm^2 s^{-1}$	$k^0_{f,h}$ cm s^{-1}
Oxazepam :														
2.0	0.72	6.28	3.68×10^{-10}	0.73	6.20	6.18×10^{-10}	0.72	6.16	3.91×10^{-7}	0.71	6.18	6.12×10^{-10}	7.58	7.20×10^{-7}
4.0	0.93	6.11	4.71×10^{-11}	0.93	6.05	9.22×10^{-11}	0.93	6.07	8.72×10^{-8}	0.95	5.98	7.22×10^{-11}	7.22	9.12×10^{-8}
6.0	1.09	6.05	7.50×10^{-12}	1.10	5.90	9.01×10^{-12}	1.06	5.85	7.17×10^{-9}	1.08	5.81	8.27×10^{-12}	7.01	9.62×10^{-9}
8.0	1.29	3.25	9.69×10^{-16}	1.26	3.00	9.82×10^{-17}	1.27	3.10	6.82×10^{-14}	1.14	3.04	9.82×10^{-13}	4.28	9.10×10^{-14}
10.0	1.45	2.05	1.89×10^{-17}	1.35	1.95	9.70×10^{-18}	1.47	1.99	5.90×10^{-15}	1.32	1.98	5.52×10^{-17}	3.90	8.80×10^{-15}
12.0	1.58	1.77	6.98×10^{-19}	1.59	1.70	1.10×10^{-20}	1.59	1.75	9.11×10^{-18}	1.59	1.71	9.98×10^{-19}	3.42	1.56×10^{-17}
Lorazepam :														
2.0	0.71	6.15	3.82×10^{-9}	0.71	6.10	5.72×10^{-9}	0.71	6.05	4.12×10^{-6}	0.70	6.01	8.65×10^{-9}	7.32	8.71×10^{-6}
4.0	0.92	6.03	4.94×10^{-10}	0.93	5.90	7.28×10^{-10}	0.89	5.97	5.81×10^{-7}	0.90	5.93	7.81×10^{-10}	7.05	7.80×10^{-7}
6.0	1.03	5.88	1.96×10^{-11}	1.06	5.88	5.47×10^{-11}	1.04	5.89	1.68×10^{-9}	1 03	5.75	5.33×10^{-11}	6.91	4.77×10^{-9}
8.0	1.24	3.11	6.82×10^{-15}	1.23	2.95	1.02×10^{-15}	1.22	3.05	8.17×10^{-13}	1.11	2.94	9.01×10^{-12}	4.16	1.12×10^{-12}
10.0	1.38	2.81	7.63×10^{-16}	1.38	1.70	1.10×10^{-17}	1.39	2.92	2.82×10^{-14}	1.26	2.69	3.18×10^{-15}	3.75	5.62×10^{-14}
12.0	1.54	2.15	1.89×10^{-18}	1.55	1.62	9.94×10^{-19}	1.57	2.18	7.14×10^{-16}	1.56	2.02	1.03×10^{-18}	3.28	9.56×10^{-16}

On the basis of the results obtained as well as from the literature, the following mechanism may be proposed for the electrochemical reduction behaviour of oxazepam and lorazepam in the entire pH zones :



where R = H for oxazepan
R = Cl for lorazepam

Analysis : In the present study, the experimental data of differential pulse polarography are used to work out analytical procedures for the estimation of the title compounds in pure form and in their dosage forms. The polarographic waves obtained in acidic media (pH 2.0–6.0) are well resolved and are used for the analysis employing both calibration and standard addition methods. Using the calibration method, the peak height is found to be linear over the concentration range 1.0×10^{-5} to 1.5×10^{-7} *M* for oxazepam and 2.5×10^{-5} to 2.0×10^{-7} *M* for lorazepam, and the respective lower detection limits are found to be 1.25×10^{-7} and 1.85×10^{-7} *M*.

Recommended analytical procedure : Stock solutions (1.0×10^{-5}*M*) of the two compounds are prepared by dissolving the appropriate amount of the electroactive species in DMF. 1.0 ml of the unknown solution is transferred to a polarographic cell and made up with 9 ml of the supporting electrolyte and then deoxygenated with pure nitrogen gas for 10 min. After recording the polarogram, small increments (0.2 ml) of standard solution are added and the polarograms are recorded after each addition under similar experimenttal conditions. The optimum conditions for the analytical determinations of title compounds are given in Table 2.

TABLE 2

	Peak Potential V	pH of supporting electrolyte	Pulse amplitude mV	Drop time s
Oxazepam	–0.95	4.0	50	2
Lorazepam	–0.90	4.0	50	2

The relative standard deviation and correlation

TABLE 3 – ASSAY OF OXAZEPAM AND LORAZEPAM DOSAGE FORMS BY DIFFERENTIAL PULSE POLAROGRAPHY

Pulse amplitude = 50 mV, Drop Time = 2 s

Sample (Tablet)	Labelled amount mg	Amount found mg	Recovery %	Standard deviation
Oxazepam				
Serepax	15	14 76	98 40	0 021
Serepax	10	9 78	97 80	0 040
Serenid Forte (D)[a]	30	29 46	98 20	0 015
Serenal[a]	15	14 79	98 60	0 016
Lorazepam				
Larpose	1	0 98	98 00	0 037
Larpose	2	1 972	98 60	0 016
Activan	1	0 998	99 80	0 012
Activan	2	1 98	99 00	0 010
Trapex	2	1 988	99 40	0 015

[a]Capsule

coefficient (for 10 replicants) are found to be 1.4% and 0.997 for oxazepam, 1.29% and 0.998 for lorazepam respectively.

Analysis of pharamaceutical dosage forms : The above described procedure is successfully utilised for the determination of the title compounds in various pharmaceutical formulations.

Twenty tablets or capsules are weighed and the average mass per tablet or capsule is measured. A portion of the finely ground sample is taken that according to label (Indian Pharmacopoeia, I.P.) would result in an approximately 10^{-2} *M* solution. The accurately weighed sample is stirred for 20 min in 50 ml of DMF. The solution is filtered and the filtrate is made upto 100 ml with DMF. By dissolving required quantity of this stock solution with the supporting electrolyte, the desired concentration of the test solution is obtained. The assay results of the various dosage forms investigated are given in the Table 3. Comparison of the values obtained by the spectrophotometric method described in the British Pharmaceutical Codex[7] for pure drugs shows that the results obtained for the pure drugs by both the methods are in close agreement. Therefore, the differential pulse polarographic method as described here can safely be used as an alternative to the official spectrophotometric method. The proposed method involves a cheaper instrument than those used in hplc and is applicable for the routine quality control of benzodiazepines in phamaceutical industry.

Experimental

Pharmaceutical grade (100% pure) oxazepam and lorazepam were obtained from Wyeth Laboratories Limited and Cipla Limited (India) respectively. Standard solutions were prepared in double-distilled dimethylformamide.

For recovery studies, tablet and capsule formulations were prepared according to the manufacturer's specifications.

The universal buffers of pH range 2.0–12.0 were prepared by using 0.2 *M* boric acid, 0.05 *M* citric acid and 0.1 *M* trisodium-orthophosphate.

The test solutions were prepared by mixing required quantity of the stock solution and making up with the supporting electrolyte to the required volume to get the concentration and deaerated with oxygen-free nitrogen gas for 10 min and polarographed at 28°.

The details of the equipment used for the electro-reduction techniques and the DME and HMDE characteristics have been described elsewhere[8].

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