AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Voltammetric Reduction Behaviour and Analysis of Lorazepam In Pharmaceutical Formulations

Chenna Rohini Kumar Putta[*1], Ramakrishna Naidu Gurijala[2], Balaji Kodigutta[1], Sridevi Chadive[3]and Suresh Reddy Cirandur[1]*

1 Department of Chemistry, S.V. University, Tirupati – 517502
2 Department of Environmental Sciences, S.V. University, Tirupati - 517502
3 Department of Chemistry, S.P.W. Degree and PG College, Tirupati- 517502

ABSTRACT

The voltammetric reduction behaviour of Lorazepam has been studied by using d.c polarography, cyclic voltammetry (CV) and differential pulse polarography (DPP) in Britton-Robinson buffers of the pH ranging from 2.0 to 12.0. DPP has been developed for the quantitative estimation of Lorazepam in different pharmaceutical formulations (Tablets) without any prior separation using standard addition method. A single step reduction wave/peak is found to be irreversible and diffusion controlled. Kinetic parameters such as transfer coefficient, diffusion coefficient and heterogeneous forward rate constant values are evaluated and reported. On the basis of the experimental results, a reduction mechanism is proposed for Lorazepam.

Key words: Lorazepam, Reduction behaviour, Mechanism, Analysis, Pharmaceutical formulations

*Corresponding Author Email: csrsvu@gmail.com
Received 29 November 2013, Accepted 01 December 2013

Please cite this article in press as: Reddy S *et al.*, Voltammetric Reduction Behaviour and Analysis of Lorazepam In Pharmaceutical Formulations. American Journal of PharmTech Research 2013.

INTRODUCTION

Benzodiazepines, an extensively studied class of psychotherapeutic drugs, are used for their tranquilizing and sedative-hypnotic properties.[1] Due to their hypnotic, sedative and anticonvulsant effects, benzodiazepines have become world-wide one of the most frequently prescribed and used anxiolytic drugs.[2]

Lorazepam [7-chloro-1,3-dihydro-3-hydroxy-5-(2-chlorophenyl) -2H-1,4-benzodiazepine-2-one] (Figure 1) is an active benzodiazepine drug with a depressant action on the central nerves system. It has anxiolytic and sedative properties which one of value in the symptomatic relief of pathologic anxiety in patients with anxiety disorders giving rise to significant functional disability, but is not considered and indicated in the management of trait anxiety.[3] It has also been shown to posses anticonvulsant activity and it is useful for the short-term relief of manifestation of excessive anxiety in patients with anxiety neurosis. It is also useful for the relief of excessive anxiety that might be present prior to surgical interventions.[4,5] Various chromatographic methods are reported in the literature for the analysis of different benzodiazepines.[6-9]

The present work deals with the electrochemical study of Lorazepam to get more information on the reduction behaviour of the compound and the electrode kinetics concerned, employing advanced electrochemical techniques. Since the application of electrochemistry to the Lorazepam drug is very limited, it is chosen to get more information on the electrode kinetics as well as reduction mechanism of azomethine (>C=N-) group, by employing advanced electrochemical techniques such as d.c. polarography, cyclic voltammetry and differential pulse polarography. A rapid, simple and sensitive differential pulse polarography method has been applied to determine the Lorazepam in different pharmaceutical formulations(Tablets).

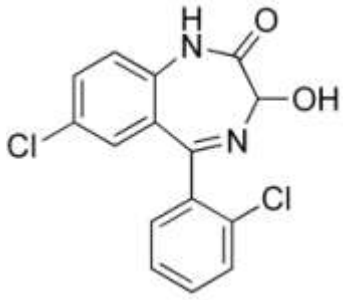


Figure 1. Structure of Lorazepam

MATERIALS AND METHOD

Polarographic Analyzer model 364 supplied by Princeton Applied research corporation (U.S.A)coupled with BD 8 Kipp and Zonen x-t recorder is used to record all the d.c. polarograms

in the present study. Metrohm unit E 506 polar cord coupled with E 612 VA-scanner, E 648 A controller and a digital electronics x-y/t recorder are used for cyclic voltammetry and differential pulse polarographic measurements.

The dropping mercury electrode (DME) with the flow rate of 2.73 mgs^{-1} and area of 0.0223 cm^2 is used in d.c. polarography and differential pulse polarography. The hanging mercury drop electrode (HMDE) of area 0.02323 cm^2 is used as the working electrode for cyclic voltammetry. In controlled potential electrolysis, mercury pool electrode is used as working electrode. Double distilled mercury is used for the working electrodes in all the experiments. Saturated calomel electrode is used as reference electrode in controlled potential electrolysis and d.c. polarography and Ag/AgCl(s), Cl^- electrode in differential pulse polarography and cyclic voltammetry. Platinum electrode is used as an auxiliary electrode in all the techniques employed to complete electrolytic circuit. The pH measurements are taken with model LI 120 Elico digital pH meter. All experiments are carried out at the temperature 25±1^oC.

The sample, Lorazepam is obtained from Sigma chemical company, U.S.A. and is used without further purification. Universal buffers ranging from pH 2.0 to 12.0 are used as supporting electrolytes and they are prepared by using 0.2 M boric acid, 0.05 M citric acid and 0.1 M tri sodium ortho phosphate. The chemicals used were of Annular grade. Stock solution of Lorazepam is prepared by dissolving the required amount in dimethyl formamide (DMF) and making up with the supporting electrolytes to obtain the desired concentration. The test solution is deoxygenated by purging with pure nitrogen gas for 5 minutes and then the voltammogram is recorded.

RESULTS AND DISCUSSION

Characterization of the wave/peak

Lorazepam is found to exhibit a single cathodic wave/peak in all the buffer systems studied over the entire pH range 2.0 to 12.0. Typical voltammograms are shown in Figures. 2-4. This wave/peak represents the saturation of the azomethine group (>C=N-) in a two electron reduction process.

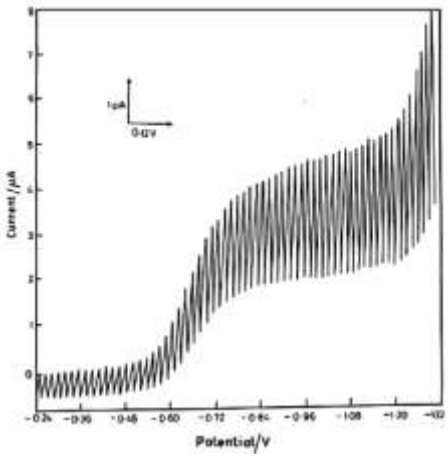


Figure 2. Typical d.c. polarogram of lorazepam in pH 2.0, Concentration: 0.5 mM, Drop time: 3 Sec

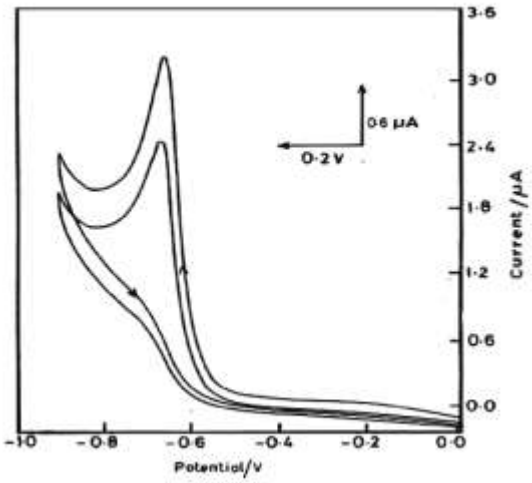


Figure 3.Typical cyclic voltammogram of lorazepam in pH 2.0, Concentration: 0.5 m M, Scan rate: 40 mVs^{-1}

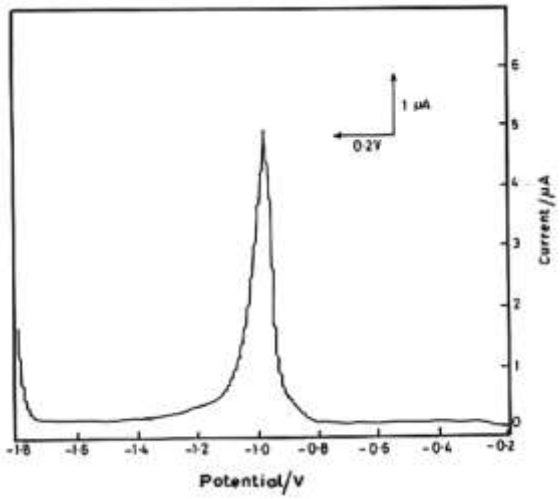


Figure 4.Typical Differential pulse polarogram of lorazepam in pH 12.0, Concentration: 0.5mM, Drop time:2sec.

Nature of the electrode process

Variations of i_d and i_p with the square root of the height of mercury column and scan rate are perfectly linear and passing through origin meaning that the electrode process is diffusion controlled without any adsorption complications. The reduction process is found to be irreversible as seen from the variation of reduction potentials with concentration. The $E_{1/2}$ and E_p values are observed to show a regular increase in the negative direction with increase in pH of the buffer solution indicating the proton involvement in the electrode process.

Identification of product

The total number of electrons involved in the electrode reduction of lorazepam is found to be two as determined by millicoulometric technique in acidic (pH 2.0) and basic (pH 10.0) media. The wave/peak heights observed are found to be almost equal in all the techniques in the entire pH range confirming the 'n' value as two. Various workers[10,11] have also studied the reduction of azomethine group in benzodiazepines and demonstrated the saturation of >C=N- group is a two electron reduction process. Lorazepam is also found to follow the usual reduction of azomethine group. Controlled potential electrolysis is carried out in pH 2.0 at -0.70 V vs. SCE and the reduction product is identified as the corresponding saturated derivative of the azomethine group (>CH-NH-).

Kinetic data

Kinetic parameters such as transfer coefficient, diffusion coefficient and heterogeneous forward rate constant are evaluated and reported in Tables 1-3. The adsorption free nature of the electron process is clearly evidenced from the nearly equal diffusion coefficient values obtained from all the techniques. The diffusion coefficient values are seen to gradually decrease which account for the decrease in diffusion current with increase in pH due to non-availability of protons. The heterogeneous forward rate constant values are observed to be low in highly basic media (pH ≥ 10) when compared to that obtained in acidic media, since the proton availability is less in the former case. With increase in pH, the forward rate constant values are found to decrease as expected in all the techniques.

Table . 1 Typical d.c. polarograpic data of Lorazepam, Concentration: 0.5 mM, Drop time :3 Sec.

pH	$\frac{-E_{1/2}}{V}$	$\frac{i_d}{\mu A}$	$\alpha.n_a$	$\frac{D\times10^5}{cm^2\,s^{-1}}$	$\frac{k^{\circ}_{f,h}}{cms^{-1}}$
2.0	0.64	3.85	0.90	6.02	5.10×10^{-10}
4.0	0.73	3.70	0.91	5.71	3.11×10^{-12}
6.0	0.81	3.60	0.91	5.26	2.52×10^{-12}

8.0	0.93	3.50	0.88	4.97	2.74×10^{-14}
10.0	1.02	3.35	0.83	4.76	8.67×10^{-15}
12.0	1.13	3.00	0.80	4.55	4.45×10^{-16}

Table . 2 Typical cyclic voltammetic data of Lorazepam, Concentration: 0.5 mM, Scan Rate : 40 mVs^{-1}

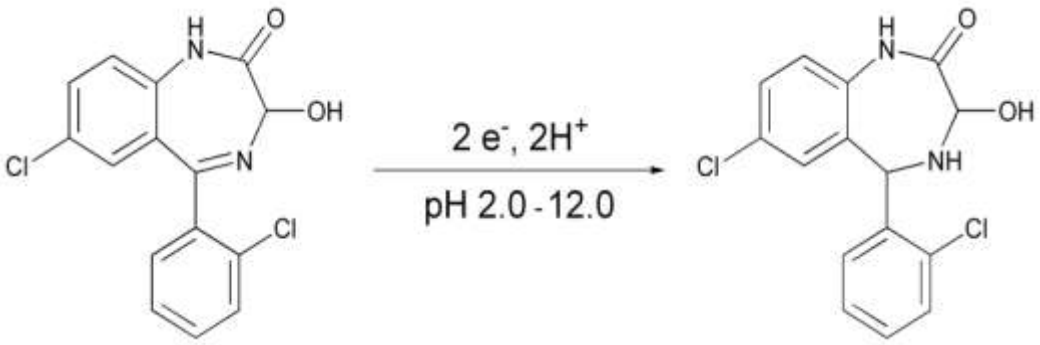
pH	$\frac{-E_p}{V}$	$\frac{i_p}{\mu A}$	$\alpha.n_a$	$\frac{D\times10^5}{cm^2\ s^{-1}}$	$\frac{k^\circ_{f,h}}{cms^{-1}}$
2.0	0.64	3.45	0.63	6.10	2.11×10^{-10}
4.0	0.74	3.35	0.65	5.72	8.90×10^{-12}
6.0	0.82	3.20	0.69	5.04	3.17×10^{-13}
8.0	0.93	3.15	0.71	4.50	1.30×10^{-15}
10.0	1.03	3.05	0.61	4.35	3.21×10^{-16}
12.0	1.14	2.95	0.61	4.50	2.05×10^{-16}

Table . 3 Typical differential pulse polarographic data of Lorazepam, Concentration: 0.5 mM, Drop time : 3 Sec

pH	$\frac{-E_m}{V}$	$\frac{i_m}{\mu A}$	$\alpha.n_a.$	$\frac{D\times10^5}{cm^2\ s^{-1}}$	$\frac{k^\circ_{f,h}}{cms^{-1}}$
2.0	0.61	5.60	0.65	5.20	8.99×10^{-11}
4.0	0.69	5.55	0.59	5.10	2.19×10^{-12}
6.0	0.78	5.35	0.61	4.92	6.19×10^{-14}
8.0	0.90	5.15	0.60	4.65	5.38×10^{-15}
10.0	0.99	5.00	0.63	4.57	1.59×10^{-16}
12.0	1.03	4.95	0.59	4.39	4.08×10^{-16}

Electrode mechanism

Based on the results obtained from different techniques, the electrochemical reduction of Lorazepam in which the azomethine group is reduced with the addition of two electrons can be proposed as follows in Scheme 1:



Scheme 1. Electrode Mechanism of Lorazepam

Analysis

Differential pulse polarography is found to be a suitable technique for the analysis of Lorazepam due to its high sensitivity and resolution. The best defined differential pulse polarographic peaks for the analytical purposes are obtained at $2 \leq pH \leq 6$, because, with solutions of greater

alkalinity (pH > 8), the reduction of azomethine group is not easily facilitated owing to the non-availability of protons. The above technique is used to the determination of Lorazepam in solutions over the concentration range 1.0 x 10^{-4} M to 1.4 x 10^{-6} M ; the calibration plot between peak height and concentration is found to be linear over that range in pH 2.0. The relative standard deviation calculated by applying the recommended procedure to 10 solutions containing the compound at a concentration of 1.5 x 10^{-5} M is 1.1%. Correlation coefficient is found to be 0.989.

The standard solution is prepared by dissolving the required quantity of lorazepam in the solvent DMF to get a concentration of 1.0 x 10^{-4}M. A different pulse polarogram is recorded after deareation of the solution (1ml of solution + 9 ml of supporting electrolyte) with oxygen free nitrogen for 15 min. After recording the polarogram, an exact known quantity of the standard solution is added to the sample solution in the cell and a polarogram is again recorded under identical conditions. The concentration of the unknown electro active species is calculated by using eq. (3.29). The optimum conditions for the analytical determination of Lorazepam in pH 4.0 are found to be a drop time of 2 sec and a pulse amplitude of 50 mV. The relative standard deviation is found to be 2.1% (10 replicants). The correlation co-efficient is seen to be 0.972 in this method.

Lorazepam can be successfully determined in different pharmaceutical formulations (Acivan and Tavor) without any prior separation by using the above mentioned procedure. Table 4 gives assay of the results obtained from the analysis of various tablets in pH 4.0. The recovery obtained with this proposed method is seen as 99.1 – 99.85 %.

Table .4 Assay of Lorazepam tablets by differential pulse polarography in pH 4.0 Pulse amplitude : 50 mV, Drop time : 2 Sec.

Sample	Labeled amount (mg)	Amount found (mg)	Recovery %	Standard deviation
ACIVAN	5.0	4.95	99.22	0.023
ACIVAN	10.0	9.98	99.85	0.018
TAVOR	2.0	1.97	99.10	0.014
TAVOR	4.0	3.96	99.45	0.027

CONCLUSION

The work describes the electrochemical behaviour of Lorazepam based on the reduction of the azomethine group at dropping mercury electrode and hanging mercury drop electrode. The recovery result shows that differential pulse polarography is a simple, reliable and inexpensive method for the determination of Lorazepam in formulations. The main advantage of the proposed

method over the other ones is that the excipients do not interfere and a separation procedure is not necessary.

ACKNOWLEDGMENT

We thank University Grants Commission (UGC), New Delhi, INDIA for providing financial support through RFSMS (Research Fellowship in Sciences for Meritorious Students) Fellowship (F.4-1/2013/SVUCS/CHMY/UGC-RFSMS/2012-13/PCRK) awarded under UGC-BSR program.

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