Electro chemical study and polarographic assay of Clonazepam formulations

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ABSTRACT

Electrochemical reduction behaviour of clonazepam has been carried out, by employing d.c. polarography, cyclic voltammetry, a.c. polarography and differential pulse polarography in the supporting electrolytes of the pH ranging from 2.0 to 12.0. The two - step reduction waves are found to be irreversible and diffusion controlled. Differential pulse polarography has been developed for the quantitative estimation of clonazepam in different pharmaceutical preparations without any prior separation using standard addition method. Kinetic parameters such as transfer coefficient, diffusion coefficient and heterogeneous forward rate constant are evaluated and reported. On the basis of the experimental results, a reduction mechanism is suggested for clonazepam.

Key words: Clonazepam, Electrochemical study, Mechanism, Pharmaceutical formulations.
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INTRODUCTION

Since the introduction of chlordiazepine hydrochloride in 1960[1] a large number of 1,4-benzodiazepine compounds have been investigated as tranquilisers, hypnotics, sedatives and antidepressants[2]. Clonazepam (1,3-dihydro, 7-nitro, 5-(2-chlorophenyl)-2H, 1,4-benzodiazepin-2-one) (Figure.1) has more pronounced hypnotic action than other benzodiazepines and has proved to be a useful replacement for the barbiturate hypnotics[3]. Because of their relative freedom from toxic effects in the usual doses or at over dose levels and from interactions with other drugs, they may be preferred to barbiturates or non-barbiturate hypnotics.
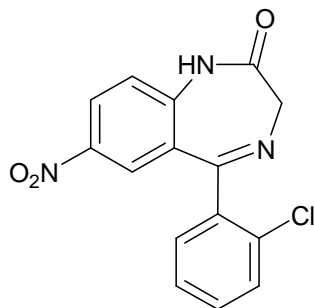
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Figure 1: Structure of Clonazepam

Studies of the electrochemical reduction and determination of this compound have not been reported. The purpose of this work is to establish the experimental conditions that permit the study of the electrochemical behaviour of clonazepam by using advanced electrochemical techniques such as d.c. polarography, cyclic voltammetry, a.c.polarography and differential pulse polarography and its determination in pharmaceutical formulation by differential pulse polarography.

MATERIALS AND METHODS

Polarographic Analyer 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 polarecord coupled with   E 612 VA-scanner, E 648 VA-controller and a digital electronics x-y/t recorder are used for cyclic voltammetry, a.c. polarography, 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, a.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 a.c. polarography, 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 28±1° C. The sample, clonazepam was obtained from sigma chemical company, U.S.A. and was 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 trisodium ortho phosphate. The chemicals used were of Analar grade. Stock solution of clonazepam was 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 was deoxygenated by purging with pure nitrogen gas for 5 minutes and then the voltammogram was recorded. A 0.02 % aqueous solution of Triton X- 100 was used to eliminate the polarographic maxima encountered throughout the polarogram.

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RESULTS AND DISCUSSION

Clonazepam is found to give two cathodic waves/ peaks in the entire pH range 2.0 to 12.0 (Figures. 2 to 5) and two well separated waves/peaks of almost equal heights in the pH range 2.0 to 6.0. The first wave/peak is assumed to be due to the consumption of four electrons in the reduction of 7 – nitro substituent to hydroxylamine and the second to the consumption of four electrons in the simultaneous reduction of the C = N group and hydroxylamine (formed as an intermediate in the reduction of the nitro group), as evidenced by the similar wave heights of the two reduction waves (Figure.2). However, in alkaline solutions, i.e.. buffer systems with pH 8.0, the identical four electrons nitro group reduction is followed by a two electron reduction of the azomethine (C=N) group. It is evident from the fact that in alkaline solution the second wave is half the height of the first wave, that the hydroxylamine is not getting reduced due to insufficient protanation (Figure.5).
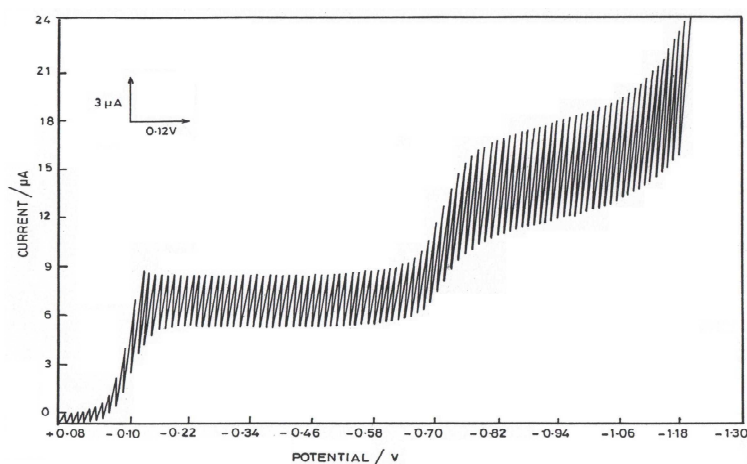

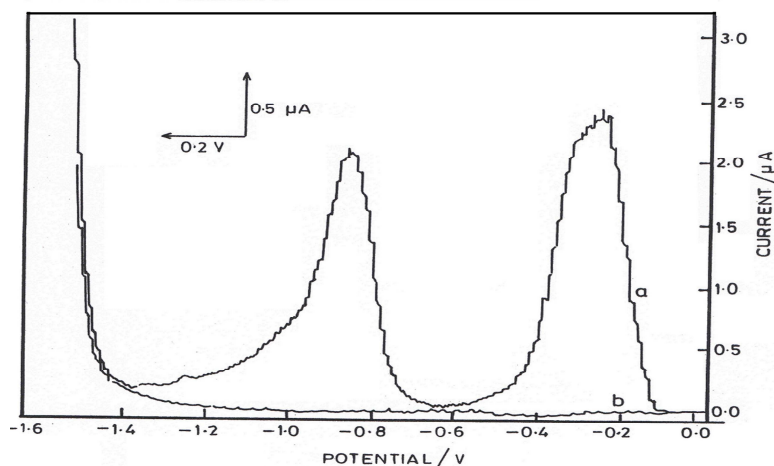**Figure 2: Typical d.c.polarogram of clonazepam in pH 2.0 Concentration: 0.5 mM Drop time: 3s**

Figure3: Typical a.c.polarogram of clonazepam in pH 4.0 Concentration:0.5 mM Drop time : 3sec. a- a.c.peak, b - base line
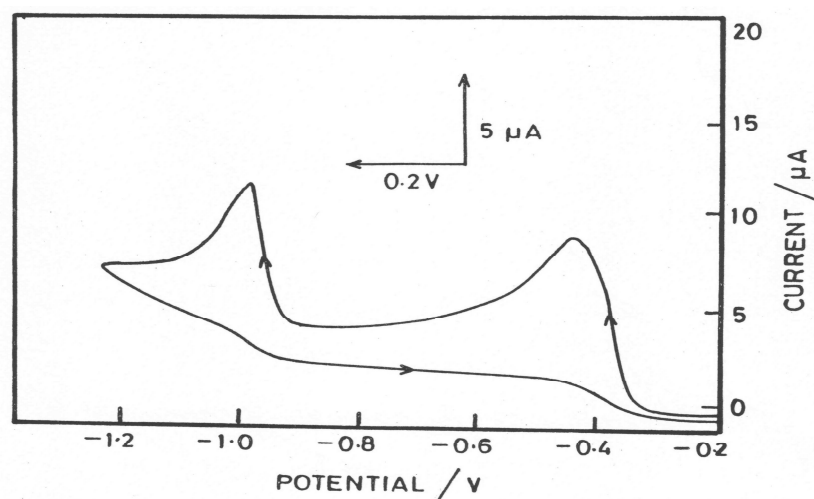
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Figure 4: Typical cyclic voltammogram of clonazepam in pH 6.0 Concentration: 0.5 mM Scan rate: 40 mVs^{-1}
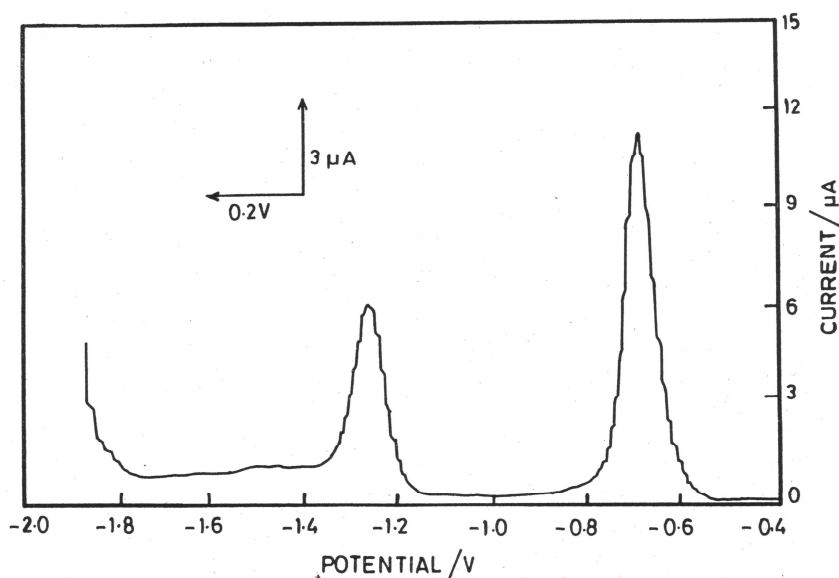

Figure 5: Typical differential pulse polarograms of clonazepam in pH 12.0 Concentration: 0.5mM Drop time: 2 sec

The diffusion controlled and adsorption free nature[4] of both of the waves/peaks are evidenced from the linear plots i_d vs $h^{1/2}$, i_p vs $v^{1/2}$ and i_m vs. $t^{2/3}$ passing through origin. In a.c. polarographic measurements the base current is not seen to depress before and after the a.c. peak also confirming the adsorption free nature of the electrode process. The experimental constancy of the current function $i_p / cv^{1/2}$ with scan rate (v) has shown the electrode process to be free from any kinetic complications. The irreversible nature of both of the waves/peaks is confirmed by log plot analysis[5] and for the each reduction process $E_{d.c}$ is plotted against [log i/i_d-i – 0.546 log t] and it was found that the value of the slopes of the above plots appreciably exceed 54.2/n mV.

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Further $E_{1/2}$, E_p, E_s and E_m are found to be shifted towards more negative potentials with increase in clonazepam concentration.[6, 7] The plot of i_m vs. $1-\sigma/1+\sigma$ is found to be non – linear according to the irreversible nature of the electrode process[8].

The variation in the proportion of the solvent (DMF) from 20% to 60% causes a decrease in i_d and i_p and the shift in $E_{1/2}/E_p$ towards more negative potentials. The decrease in diffusion current may be partly due to an increase in the viscosity of the medium. The shift in $E_{1/2}/E_P$ may be ascribed to a decrease in absorbability and hence surface concentration of the depolariser with an increase in the percentage of non–aqueous solvent in the aqueous–organic mixture. A decrease in surface concentration would retard the electrode process resulting in a decrease in $E_{1/2}/E_p$ and i_d/i_p.

The determination of the number of electrons involved in the electrode processes has been carried out by millicoulometric method. In pH 2.0. The number of electrons involved in the electrode reduction is found to be four for both the first and second waves and in pH 12.0, it is found to be four for the first wave and two for the second wave. Comparison of the heights as well as the diffusion currents of first and second waves in acidic and basic media also confirms the same. The reduction of nitro – group as well as azomethine group in different supporting electrolytes have already been discussed extensively by different workers.[9, 10] Here also the title compound is observed to follow the usual reduction of nitro and azomethine groups. Controlled potential electrolysis is carried out in pH 2.0 and in pH 12.0 at -0.8 V and -1.3 V Vs. SCE respectively and products are identified as corresponding amine and hydroxylamine. The amino compound formed in acidic solutions is isolated and is confirmed by I.R. spectral studies (Nujol, 3500 cm^{-1}. 1650 cm^{-1}) as shown in Figure.6.

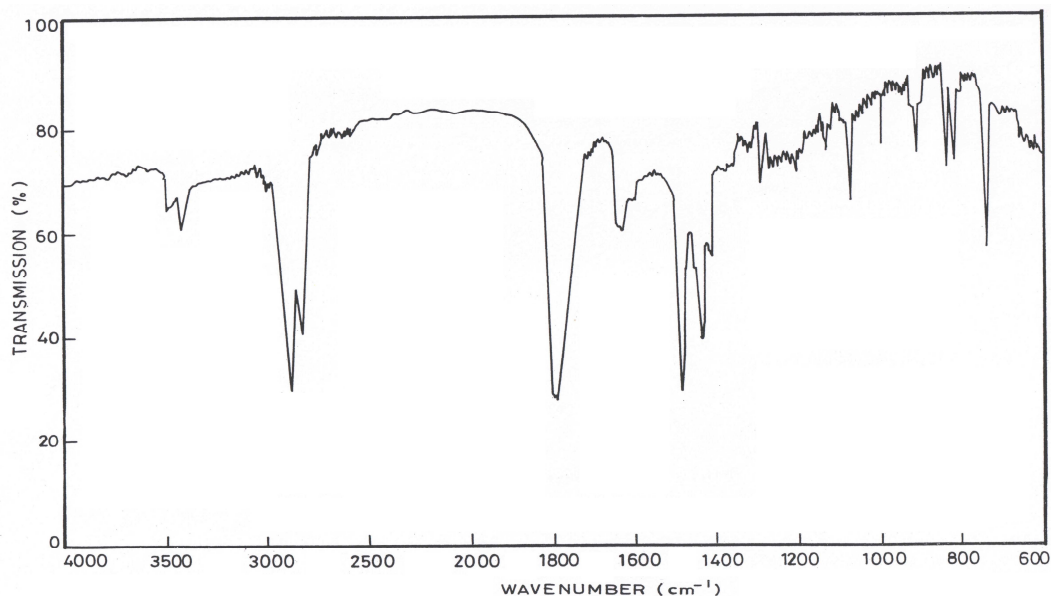Typical kinetic parameters evaluated from different techniques are presented in Table 1.

Figure 6: I.R. spectra of the electrolysis product of clonazepam

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The variation of diffusion current and peak current with the pH of the supporting electrolyte influences the diffusion coefficient values also to vary in the same manner. The reason for slight decrease in D values with increase in pH may be attributed to the non availability of protons with increase in pH. Diffusion coefficient values obtained from all the techniques are seen to be in good agreement due to the adsorption free nature of the electrode process. The heterogeneous forward rate constant values obtained for the reduction of nitro group are found to be high when compared to that of azomethine group since the nitro group reduction is facile as evidenced from the less negative potentials.

The difficulty associated with the reduction of azomethine group is also evidenced from the low rate constant values. $K^o_{f,h}$ values are observed to decrease with increase in pH of the buffer solution due to the shift of reduction potentials to more negative values with increase in pH, possibly an increase in pH increases the dissociation constant of the protonated species and these factors affect the protonated rate and consequently the reduction potentials are shifted to more negative values. The standered rate constants obtained in a.c. polarography are found to be high when compared to other techniques because the rate constants are evaluated at standard potentials in a.c. polarography whereas in other techniques the rate constants are calculated at potentials E=O. The magnitude and the nature of the charge transfer contribution to frequency in a.c.polarography also play its role in this connection.

Electrode mechanism
On this basis of the results obtained, the following reduction mechanism may be proposed for clonazepam in different pH zones:
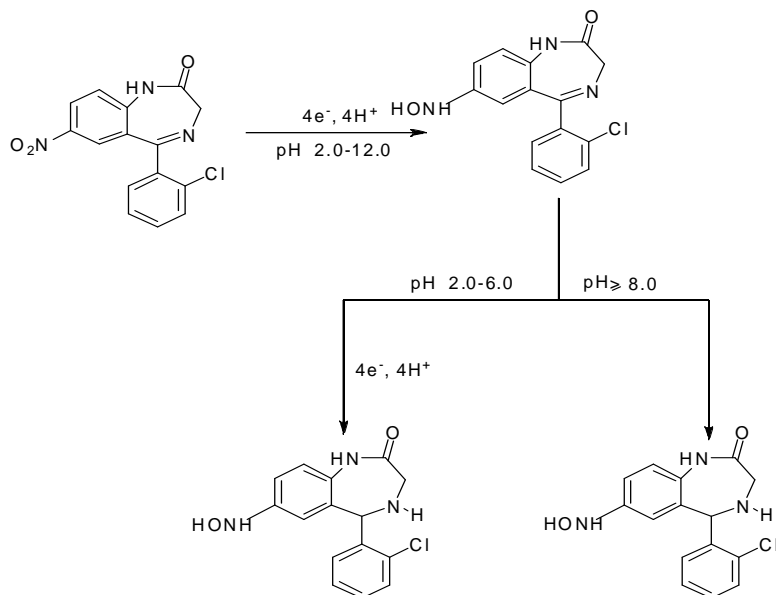
Scheme 1: Electrode Mechanism of Clonazepam

Table 1: Typical electrochemical data for clonazepam Concentration: 0.5 mM

pH of the supporting electrolyte	D.C.Polarography (Drop time 3 sec)			cyclic voltammetry (Scan rate 40 mV)			A.C.Polarography (Drop time 3 sec)			Differential Pulse Polarography (Drop time 2 sec)		
	$-E_{1/2}/V$ cm s^{-1}	D x10^5/ cm^2 s^{-1}	$K^0_{f.h}$/	$-E_p/V$ s^{-1}	D x10^5/ cm^2 s^{-1}	$K^0_{f.h}$/ cm	$-E_s/V$	D x10^5/ cm^2 s^{-1}	K_s/ cm s^{-1}	$-E_m/V$ s^{-1}	D x10^5/ cm^2 s^{-1}	$K^0_{f.h}$/ cm
2.0	a) 0.08	2.69	1.23×10^{-4}	0.10	2.78	1.73×10^{-4} 0.70	0.08	2.65	1.89×10^{-2}	0.07	2.67	1.79×10^{-4}
	b) 0.69	2.60	4.37×10^{-9}	2.61		8.43×10^{-9}	0.70	2.50	8.32×10^{-5}	0.69	2.50	4.87×10^{-9}
4.0	a) 0.22	2.60	3.43×10^{-5}	0.27	2.60	3.99×10^{-5}	0.23	2.49	2.01×10^{-3}	0.21	2.52	1.43×10^{-5}
	b) 0.90	2.47	5.49×10^{-10}	0.92	2.53	5.75×10^{-11}	0.89	2.29	4.45×10^{-7}	0.89	2.40	2.53×10^{-9}
6.0	a) 0.43	2.17	7.37×10^{-6}	0.46	2.23	2.18×10^{-6}	0.40	2.01	7.43×10^{-4}	0.43	2.20	1.88×10^{-6}
	b) 1.01	2.03	1.33×10^{-11}	1.04	2.10	1.81×10^{-12}	1.03	1.97	8.48×10^{-8}	1.03	2.10	5.45×10^{-10}
8.0	a) 0.51	2.09	2.93×10^{-8}	0.52	2.10	6.33×10^{-7}	0.50	1.95	2.83×10^{-5}	0.54	2.00	5.88×10^{-8}
	b) 1.13	1.42	1.88×10^{-13}	1.13	1.34	2.94×10^{-13}	1.10	1.35	5.55×10^{-10}	1.10	1.43	6.95×10^{-12}
10.0	a) 0.62	1.87	4.01×10^{-9}	0.65	1.98	1.63×10^{-9}	0.61	1.83	2.01×10^{-6}	0.61	1.87	6.81×10^{-9}
	b) 1.18	1.08	8.81×10^{-15}	1.20	1.10	9.33×10^{-15}	1.17	1.02	3.14×10^{-12}	1.20	1.01	2.35×10^{-14}
12.0	a) 0.69	1.82	4.55×10^{-10}	0.70	1.89	5.39×10^{-10}	0.68	1.80	8.71×10^{-7}	0.70	1.69	6.01×10^{-10}
	b) 1.23	1.00	6.11×10^{-18}	1.27	1.10	1.25×10^{-18}	1.23	0.97	5.59×10^{-13}	1.27	0.93	5.11×10^{-17}

a) First Wave/Peak   b) Second Wave / Peak

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Analysis

Differential pulse polarography has been employed in the present investigation for the analysis of clonazepam using both calibration and standard addition methods. Two polarographic peaks obtained in the entire pH range are well resolved and may be utilized for the analysis of clonazepam in pharmaceutical preparations. But first wave, which is responsible for the reduction of nitro group, is highly reproducible and is therefore preferred for analysis. Supporting electrolytes of $4.0 \leq pH \leq 6.0$ are found to suitable media for the analysis experiments. This technique is used to determine clonazepam in solutions over the concentration range of 1.0×10^{-5} M $– 2.4 \times 10^{-7}$ M. The calibration plot base on peak height vs. concentration is found to be linear over the above said concentration range in pH 4.0. Determination of clonazepam in pharmaceutical formulations (tablets) has been carried out using standard addition method. The assay results of clonazepam tablets are given table 2. The optimum conditions for the determination of clonazepam are found to be a drop time of 2 sec, pulse amplitude of 60mV and pH 4.0.

Table 2. Assay of clonazepam dosage form by differential pulse polarography in pH 4.0 Pulse amplitude: 60 mV, Drop time:2 sec.

Sample	labeled amount (mg)	Amount found (mg)	Recovery (%)	Standard deviation
Rivtrol	2.5	2.45	98.00	0.037
Rivtrol	5.0	4.93	98.60	0.016

CONCLUSION

The work describes the electrochemical behaviour of clonazepam based on the reduction of the nitro and 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 clonazepam in formulations. The main advantage of the proposed method over the other ones is that the excipietns do not interfere and a separation procedure is not necessary.

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