Cyclic Voltammetric Studies of Diazepam Using Glassy Carbon Electrode-Estimation of Diazepam in Pharmaceutical Samples

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Cyclic voltammetry of diazepam has been investigated in acid solution (pH 1.0) on a glassy carbon electrode. A well defined cathodic peak is observed. The interferences by the solution components on the estimation are studied. A simple, accurate, sensitive and rapid method for the determination of the drug in pharmaceutical formulations is proposed.

Key words cyclic voltammetry; glassy carbon electrode; diazepam

Diazepam (DZ), 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one, is a psychotherapeutic agent of medicinal importance in the treatment of diseases of the central nervous system. Hence, a study on the quality control of DZ is a subject of considerable importance.

Many methods have been reported in the literature for the assay of DZ, which include colorimetric,[1] chromatographic,[2] polarographic,[3] fluorimetric,[4] titrimetric[5] and HPLC[6] techniques. However, most of these methods are time consuming, involving elaborate procedures with low sensitivity. In the HPLC technique, the excipient present in the pharmaceutical formulation requires filtration using a millipore filter, which interferes with the estimation. In the colorimetric method, an element of error is introduced because of the masking of the colour by the colouring agents and degradation products with prolonged storage. The official pharmacopoeia[7] method which describes the spectrophotometric technique for the assay of DZ in dosage forms, is not specific for the intact drug and does not take into account the interference by the degradation product. Elimination of the interference from other UV-absorbing compounds such as co-formulated drugs and formulation excipients is also not possible.

In aqueous solution, DZ is slowly reduced/hydrolysed/transformed into adducts. The *in situ* generation of these products cannot be easily distinguished from the intact DZ unless tested and eliminated.

The aim of the present communication is to overcome some of the above problems and develop a simple, accurate, sensitive and rapid method for the estimation of DZ and in its pharmaceutical formulations by electrochemical reactions on a glassy carbon electrode (GCE) using the cyclic voltammetric technique.

Experimental

DZ (Ray Chemicals, India) was used after purification (mp 131—135 °C). All solutions were prepared by using AR chemicals and double distilled water. The pH of the solution was adjusted with dilute HCl/NaOH solution.

Cyclic voltammetric experiments were carried out in a single compartment glass cell of 50 ml capacity with glassy carbon (0.125 cm^2) as the working electrode. Platinum foil (0.5 cm^2) and calomel were used as counter and reference electrodes, respectively. The glassy carbon was polished with diamond paste of 1 μm particle size on a polishing table and electrochemically cleaned[8] before use. Voltammograms were recorded using a Scanning Potentiostat (EG & G 326A, U.S.A.).

Test solution containing a definite amount [1×10^{-3} M] of DZ in alcoholic-buffer (pH 1) medium was de-aerated by bubbling oxygen free AR nitrogen gas for 10 min. All experiments were repeated to ensure reproducibility. Fresh solution was used for each experiment.

Results and Discussion

Voltammograms of DZ were obtained using a solution with pH 1 at a scan rate of 5 mV/s in the potential range -0.9 to -0.4 V. A single cathodic reduction peak at -0.775 V was noticed. Experiments were conducted at different scan rates (γ). The value of the peak potential (Ep) became more cathodic on increasing the scan rate (Fig. 1) and varied linearly with $\gamma^{1/2}$ indicating the irreversibility of the reduction step. Voltammograms were recorded using the solution of DZ with different pH values (1—13) in the potential range -1.45 to -0.4 V. It is noticed that as the medium becomes alkaline, the cathodic peak shifts considerably to higher potential region showing the dependence of the reduction reaction on the pH of the medium (Fig. 2).

In order to know the products obtained during electrochemical reduction, DZ solution at pH 1 was electrolysed at -0.8 ± 0.02 V for 24 h in a two compartment cell. A major compound from the cathodic compartment was extracted and characterised as 4,5-dihydro-diazepam (reduced product). Thus the single cathodic peak of Fig. 1 is due to the reduction of DZ molecule by a two electron change as reported by earlier researchers.[9]

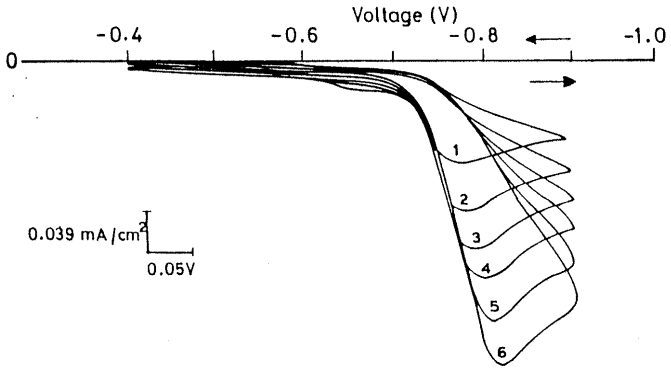

Fig. 1. Cyclic Voltammograms of DZ Obtained on Glassy Carbon in the Potential Range of -0.9 to -0.4 V as a Function of the Sweep Rate
(1) 2 mV/s, (2) 5 mV/s, (3) 10 mV/s, (4) 20 mV/s, (5) 50 mV/s, (6) 100 mV/s.

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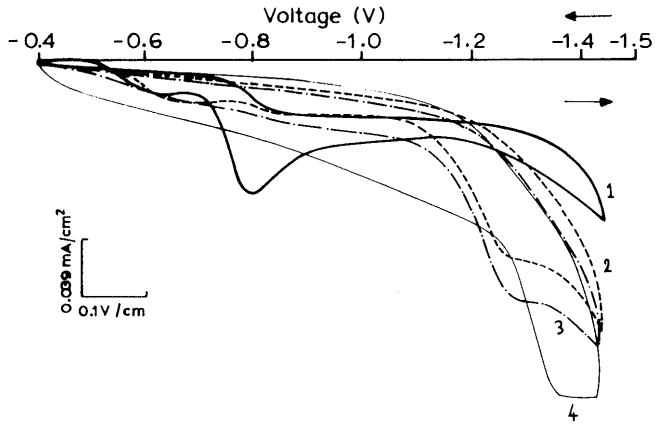

Fig. 2. Cyclic Voltammograms Obtained on Glassy Carbon from −1.45 to −0.4 V at a Scan Rate of 5 mV/s in Different pH Media

(1) pH 1.0, (2) pH 4.0, (3) pH 8.0, (4) pH 13.0.

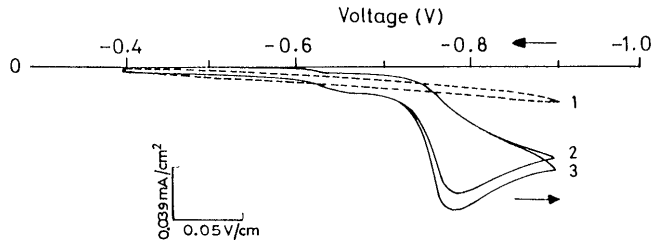

Fig. 3. Cyclic Voltammograms Obtained on Glassy Carbon in the Potential Range of −0.9 to −0.4 V at a Scan Rate 5 mV/s

(1) 1×10^{-4} M Hp, (2) 1×10^{-4} M Hp and 1×10^{-3} M DZ, (3) 1×10^{-3} M pure DZ.

Other compound in small amount was separated by preparative TLC using solvent system—benzene : ether : methanol (7.5 : 10 : 0.35) with *Rf* 0.92, and it was characterised by IR and NMR spectra:

IR (KBr) γ_{max} cm^{-1}: 1621 s (C=O), 3338 m (NH), 710 s (C–Cl).

^{1}H-NMR ($CDCl_3$) δ: 2.95 (d, 3H, Me), 6.70—7.70 (m, 8H, Ph), 8.45 (br, 1H, NH)

as benzophenone derivative of DZ, MACB (2-methylamino-5-chlorobenzophenone). Another reaction product was identified as glycine[10] with ninhydrin reagent. These are the products from the hydrolysis of the DZ molecule.

Cyclic voltammograms were obtained under comparable experimental conditions using the benzophenone derivative of DZ and glycine. In fact, the hydrolysed product (Hp) failed to give a peak in the selected potential range (Fig. 3-1), while the addition of DZ to the same solution gave a cathodic peak with a reduction in peak current (Ip) (10—15%) (Fig. 3-2) compared to pure DZ (Fig. 3-3). This may be due to surface adsorption of Hp on the electrode surface.

To know the stability of DZ solution with storage and also the effect of degraded product, if any, on the estimation of DZ, an experiment was conducted with a definite concentration of DZ at pH 1. After taking the cyclic voltammogram, the solution was stored for a longer period (24 h) and the same solution was used to get the cyclic voltammogram again. A slight decrease in cathodic peak current was noticed due to hydrolysis. This effect was also observed by earlier scientists[3] but without any explanation.

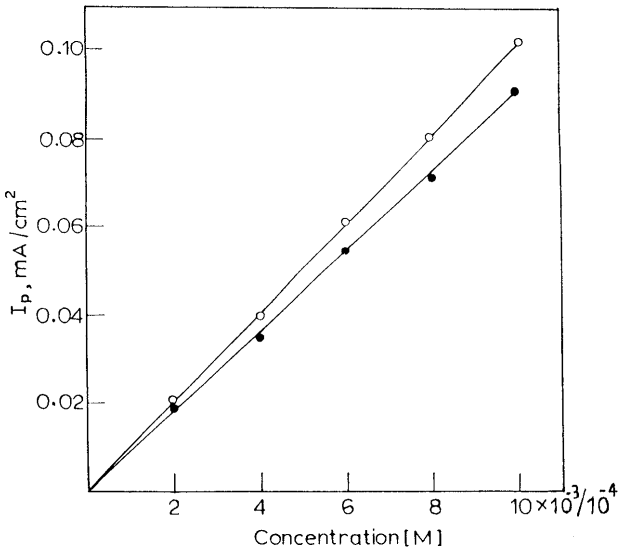

Fig. 4. A Calibration Graph of Pure DZ and DZ+Hp at a Scan Rate of 5 mV/s in pH 1.0 Using Glassy Carbon Electrode in the Potential Range of −0.9 to −0.4 V

○, 10^{-3} M DZ; ●, 10^{-3} M DZ + 10^{-4} M Hp.

Table 1. Estimation of DZ in Pure Form and in Pharmaceutical Formulations in Buffer (pH 1)

DZ and its pharmaceutical formulation	Amount claimed (mg)	Amount taken (mg)	Recovery (%) proposed method	BP method[7]
Pure DZ	—	5—20	99.8 (±0.3)	99.75 (± 1.25)
DZ tablet	05/tab	10	99.7 (±0.4)	100.00 (± 7.50)
DZ capsule	20/2 cap	10	99.8 (±0.3)	100.00 (± 7.50)
DZ injection	20/4 ml	10	99.9 (±0.2)	100.00 (±10.00)
DZ syrup	10/25 ml	10	99.7 (±0.4)	105.00 (±10.00)

Average of six determinations with standard deviations given in parentheses.

To quantify these data, experiments were carried out with different concentrations [1×10^{-3}—1×10^{-4} M] of DZ at a scan rate (5 mV/s) in HCl–KCl buffer of pH 1 at 303 K. A linear relationship between Ip value of the voltammogram and the concentration of DZ was noticed. A similar calibration graph of DZ+Hp of known concentration was obtained by recording the voltammograms immediately (Fig. 4). An unknown concentration of DZ containing Hp was also determined from this calibration plot. If Ip is the peak current of the freshly prepared pure DZ in the known solution and Ip* of DZ solution containing Hp, one can calculate the percentage of the hydrolysed product and its effect on the estimation in the test solution using the relation:

$$\frac{Ip - Ip^*}{Ip} \times 100 = Hp\%$$

This procedure was also extended to estimate DZ in various pharmaceutical formulations without prior separation of the excipients (Table 1). The proposed method is accurate, sensitive, rapid and better than the official method.[7]

To test the precision and reproducibility of the CV method, six replicate samples were analysed, each containing [1×10^{-3} M] of DZ. The values reproducible with

good precision (Table 1). The recoveries of DZ in pharmaceutical formulations are in the range 99.7—99.9%.

Recommended procedure: Pipette out 5ml of alcoholic solution containing DZ or its pharmaceutical formulation in the concentration range [1×10^{-3}—1×10^{-4} M] into a 50 ml standard flask. Add HCl–KCl buffer (pH 1) solution to make up 50 ml. Transfer entire solution to a single compartment glass cell provided with pre-cleaned glassy carbon, platinum foil and calomel electrodes. Purge nitrogen gas through the solution for 10 min. Record the cyclic voltammogram in the potential range -0.9 to -0.4 V at a scan rate of 5 mV/s. Evaluate the Ip value and estimate the amount of DZ in the given solution using the calibrated results.

Conclusion

The present studies have shed light on the interference of degradable products in the voltammetric estimation of DZ; these interferences were either overlooked by earlier workers or not addressed. Attempt has been made to estimate DZ in the presence of the interfering components.

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