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Electrochemical reduction of diazepam

L THOMAS[1], J L VILCHEZ*[1], G CROVETTO and J THOMAS

[1] Department of Analytical Chemistry, Faculty of Sciences,
Department of Physics-Chemistry, Faculty of Pharmacy, University of Granada, Granada, Spain.

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Abstract. The reduction of 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepine-2-one at the dropping mercury electrode has been investigated. The process is diffusion controlled; furthermore, the reduction wave enables the quantitative determination of the drug both in acid and basic media. The reagent captures two electrons and two hydrogen ions. A disproportionation reaction of the hydrazo-compound takes place, and amines are the final reduction products.

Keywords. Diazepam; polarography; cyclic voltammetry.

1. Introduction

Diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepine-2-one, trade names; *Valium*, *Vival* and *Stesolid*, among others), a psychotherapeutic agent extensively used as a tranquilizer (Kales and Sharf 1973; Haider 1968; Matthew *et al* 1969), is also employed as an anticonvulsive in epileptic states (Browne and Penry 1973) due to its cardiovascular effects (Rao *et al* 1973). Because of its pharmacologic importance several procedures have been outlined for the determination of the presence of the drug in different materials by chromatography (De Silva *et al* 1964), spectrophotometry (De Silva *et al* 1966) and polarography (Oelschlager *et al* 1964; Barret *et al* 1973). The particular advantage of the last method is that the analysis can be carried out in the presence of the products of acid hydrolysis. As in many other medicines its action is closely related to oxidation-reduction mechanisms. Thus the electrochemical character of the polarographic study can greatly help obtain additional information.

The polarographic activity of the 1,4-benzodiazepines has been extensively reported. Senkowski *et al* (1964) investigated the polarographic behaviour of diazepam and Oelschlager (1963) compiled data on the related compound chlordiazepoxide hydrochloride. The reduction mechanism of oxazepam (Delschlager *et al* 1970) and nitrazepam (Halvorsen and Jacobsen 1972) have been also reported, as well as other benzodiazepines. Various assays have been developed for the analysis of chlordiazepoxide, diazepam, oxazepam, medazepam, nitrazepam, bromazepam, clonazepam, flunitrazepam and clorazepam in both biological samples and pharmaceutical dosage forms (Brooks *et al* 1975).

*To whom all correspondence should be addressed.

It is the purpose of this paper to complete the study of the reduction reaction of diazepam by conventional and oscillographic polarography, and voltammetric and coulometric techniques in order to elucidate its electrochemical behaviour.

2. Experimental

The diazepam was supplied by IMPEX Quimica.

Direct current polarography: Tacussel and PO4 Radiometer. Electrodes: SCE, DME (m = 2·2 mg sec^{-1}; t = 3·92 sec), and a platinum wire.

Oscillographic polarography: Chemtrix SSP-3; SCE and dropping mercury electrode.

Cyclic voltammetry and coulometry: Amel-471. Electrodes used: SCE, working electrode for the cyclic voltammetry E410 Metrohm mercury hanging drop electrode. Controlled potential studies were carried out with a large stirred mercury pool electrode. In all the experiments the oxygen was removed by bubbling nitrogen gas (99·998% purity) through the solution. Britton–Robinson (B-R) buffers were used as supporting electrolyte solutions.

Since Cimbara and Gupta (1965) showed that the drug decomposed in aqueous solutions, we have dissolved the drug in methanol 20%/H_2O. Decomposition was negligible in this solvent. Therefore methanol-water mixtures have been used throughout. The polarographic measurements were done using a thermostated AMEL 494 cell. In all cases the temperature was maintained at 25·0 ± 0·1°C.

3. Results and discussion

3.1. *D.C. Polarography*

The diazepam reduced at the dropping mercury electrode produces a polarographic wave at different concentrations, temperature and pH values. Figure 1 shows a representative polarogram at pH = 1·81, and the half-wave potential is $E_{1/2} = -0{\cdot}705$ V.

Examination of the electrocapillary curves (figure 2), obtained for 10^{-3} M diazepam and supporting electrolyte (B-R buffer, pH = 1·81) solutions, shows a great reduction of the drop time at the reduction potential. Based on this fact we can deduce that the drug is surface-active and is strongly absorbed on the dropping mercury electrode surface.

The effect of the drop time on the limiting current was analyzed by recording polarograms of 5×10^{-4} M diazepam in B-R buffer and 20% methanol at pH = 1·81 and 11·70, at various heights ($h_{corr.}$) of the mercury column. Plots of the limiting current values (i) against height were linear for both media, and the $i \times h^{-1/2}$ value remained constant. The temperature coefficient (determined in the range 5–35°C of the d.c. current) was 1·85% per degree. All these observations indicated that the polarographic wave under the conditions described is attributable to a process mainly controlled by the electroactive substance diffusing from the solution bulk to the dropping mercury electrode surface.

The effect of pH on the intensity-potential curves was also determined. The

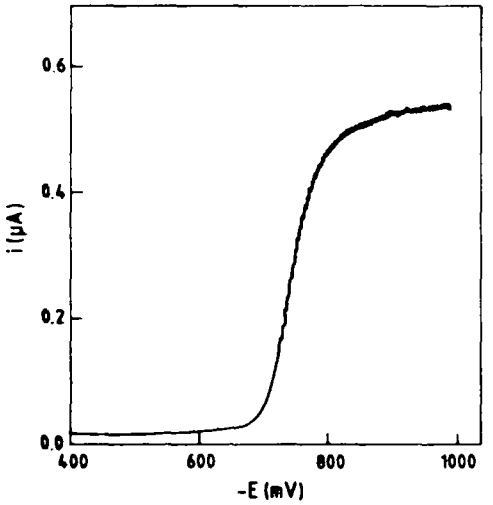


Figure 1. Polarogram of 5×10^{-4} M of diazepam dissolved in B-R buffer-methanol 20%, pH = 1·81.

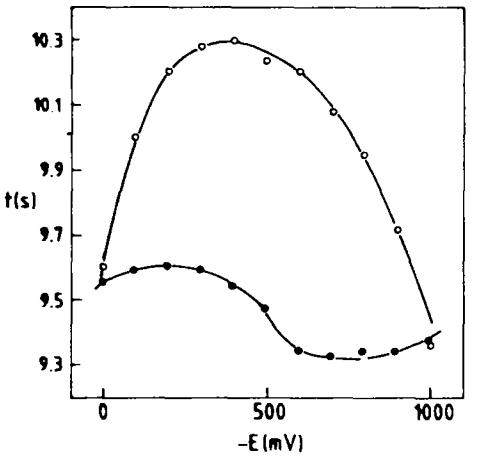


Figure 2. (-•-) Electrocapillary curve of 10^{-3} M diazepam in B-R buffer methanol 20%, pH = 1·81. (-o-) Electrocapillary curve of supporting electrolyte in B-R buffer, pH = 1·81.

half-wave potential shifts towards more negative values with an increase in electrolyte pH value indicating that hydrogen ions are consumed in the electrode reaction. There is a linear relation between both E magnitudes, and the half-wave potential, $E_{1/2} = -0{\cdot}580, -0{\cdot}060$ mV, in the pH range 1·81 – 11·88. The number of hydrogen ions, Z, involved in the electrode process is given by

$$E_{1/2}/\Delta\text{pH} = -0{\cdot}059\ Z/\alpha n_a,$$

where α is the transfer coefficient and n_a the number of electrons in the rate-controlling step of the reduction process. For the range of pH studied the αn_a

value calculated was 1·95 and the number of hydrogen ions was 1·85, indicating that two hydrogen ions are consumed in the global process.

The plots of the intensity values deduced from the diazepam polarograms at acidic (1·81) and basic (11·7) pH values versus concentrations of the active substance were linear in the 10^{-3}–$2{\cdot}5 \times 10^{-6}$ M range. The diffusion current constant (I) was 2·57 μA l mmole^{-1} mg$^{-3/2}$ sec$^{1/2}$. This value of I agrees with previously published values of similar compounds (Senkowski 1964).

3.2 *Coulometry*

The coulometric reduction at controlled potential diazepam in B–R buffer, pH 1·81, and methanol 20% was also studied. The pool mercury (used as working electrode) was maintained at $-0{\cdot}9$ V potential. The samples contained 5×10^{-5} moles of diazepam in 50 ml of solution. It appears that the reduction involves $2{\cdot}01 \pm 0{\cdot}04$ electrons, so that it can be considered as a bielectronic process.

3.3 *Cyclic voltammetry*

The cyclic electrode voltammetric curves were recorded at different scan rates (figure 3). They always show a single cathodic wave, and the anodic peak resulting from reoxidation of the reduction product is absent. The shape of the cyclic voltammetry curve is in agreement with the diagnostic criteria proposed for systems in which an irreversible chemical reaction follows a reversible change transfer (EC mechanism) (Wopschall and Shain 1967).

Voltammetric studies to evaluate the electrode reaction order and the electronic transfer coefficient were registered at different concentrations in the range of

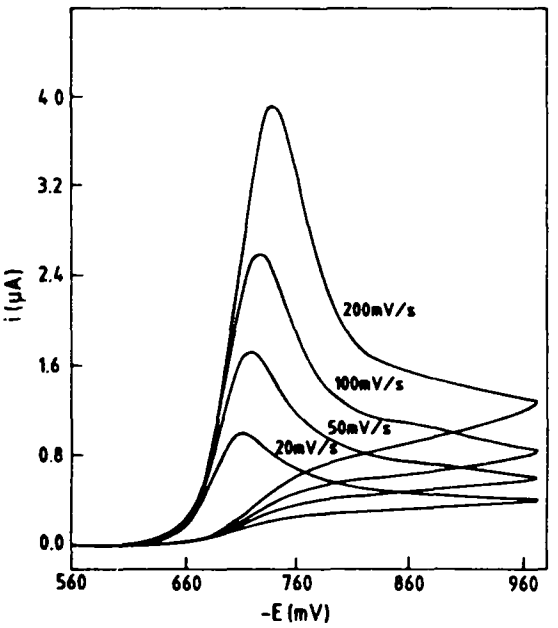


Figure 3. Cyclic voltametric curves of 3×10^{-4} M, diazepam in B-R buffer–methanol 20%, pH = 1·81.

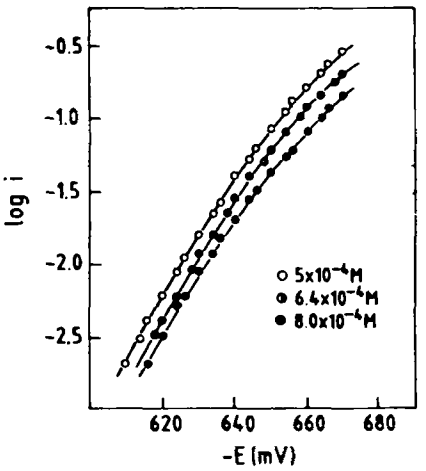


Figure 4. Representation of Tafel's equation for different reagent concentrations of diazepam.

10^{-4} M. The transfer coefficient determined from Tafel's slope (figure 4) is 1·32. Plots of log-intensity i values at $-0{\cdot}620$ V versus the log-concentration C show a linear relation. Therefore the electrode reaction order with respect to the concentration is:

$$a = \left(\frac{\partial \log i}{\partial \log C}\right)_E = 1$$

Voltammograms of diazepam at different concentrations in the range $10^{-3} - 4 \times 10^{-6}$ M as a function of the potential scan rate allowed us to deduce the function current $i_p/Cv^{1/2}$ values. In figure 5 the current function variations versus scan rate, for several concentrations of the active substance, show dependence on both factors. However, this magnitude approaches a constant "diffusion controlled" value at slow scan rates and high bulk concentrations. This is a characteristic feature for surface-active depolarizers (Wopschall and Shain 1967). At fast scan rates adsorption prevails over diffusion, but if the solution concentration increases, the influence of adsorption decreases.

3.4 *Oscillographic polarography*

The oscillopolarograms of 5×10^{-4} M diazepam, methanol 20% and B-R buffer at pH = 1·81 were recorded at different scan rates in the range between 0·5 and 10·0 V sec^{-1}. The dependence of the peak current on the square root of the scan rate of voltage can be considered as linear in the interval considered above. Extrapolation of the linear relationship does not however lead to zero current at zero voltage, but this fact has been attributed to the irreversibility of the electrode process (Delahay 1950).

Diazepam produces only one oscillopolarographic cathodic wave confirming the character of the irreversible process according to the assymetry observed between cathodic and anodic waves.

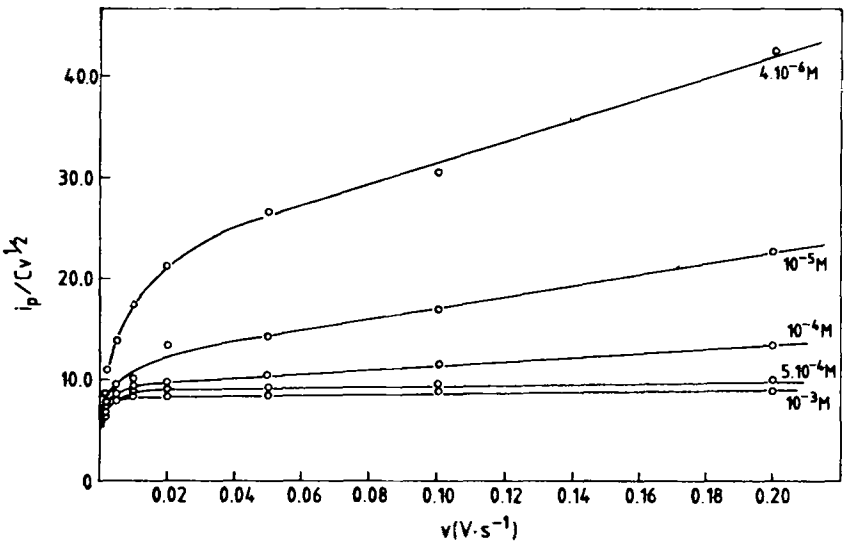


Figure 5. Current function variations versus scan rate for several concentrations of the active substance diazepam.

The dependence of the peak current on the diazepam concentration ($10^{-3} - 10^{-6}$ M) was also studied. The shape of the wave is similar to other organic substances like phenazine (Laviron 1974) which were adsorbed on the mercury drop.

The non-zero intersection point with the coordinate axis obtained by data extrapolation suggests that at $1{\cdot}7 \times 10^{-4}$ M concentration of diazepam, the electrode surface can be considered to be completely covered by a monolayer of the reaction product.

Laviron (1974) established that the interaction increases between the molecules adsorbed on the electrode surface, describing the adsorption by a certain isotherm, so that experimental features such as shifts on δ (peak width at half its height) and the peak potential (E_p), at the electroactive substance concentration, could be explained. In our case, the δ values increase with diazepam concentration, indicating that the predominance of repulsion over attraction increases between the molecules adsorbed on the surface of the mercury electrode.

The reduction peak of diazepam is well defined under the experimental conditions previously described. This may allow the quantitative determination of diazepam if the calibration curve has been established previously.

3.5 *Current-time curves*

Current-time curves were recorded at different potential values ($-0{\cdot}65$ and $-0{\cdot}90$ V). We observed that the polarographic curve of diazepam lay somewhere between them. The logarithmic analysis of the current-time curves clearly shows a linear shape. In our case, the examination of the log i versus log t plot allows us to estimate the β coefficient shift in the relation $i = k.t^{\beta}$, confirming the irreversible character of the electrode process.

4. Identification of the reduction products

In order to identify the reduction products, 50 mg of diazepam were electrolysed under the experimental conditions stated in §3.2. The system was extracted with Cl_3CH in a basic medium. The crude obtained was treated by column chromatography (2:3 hexane-ether) and 44 mg of a white powder was isolated.

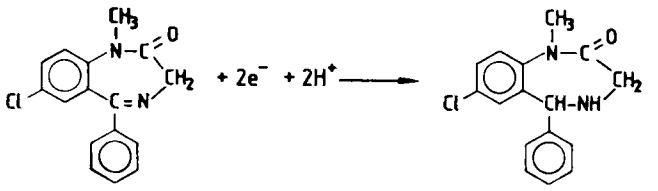
IR-The main fact observed is the appearance of a band at 3,311 cm^{-1} attributable to δ_{N-H} as well as the disappearance of $\delta_{C=N}$ at 1,650 cm^{-1} observed in the IR spectrum of diazepam.

^{1}H-NMR ($CDCl_3$): 2·70 ppm (s_{broad}, 1H; proton exchangeable with D_2O), 3·32 (*s*, 3H), 3·41 (*s*, 1H) 5·22 (*s*, 1H), 6·72 (*d*, 1H, J=2·0 Hz), 6·95–7·95 (*m*, 8H).

The spectroscopic properties as well as the elemental analysis proved that the reduction product of the diazepam is the 4–5 dihydro-diazepam. This results confirm the hypothesis proposed by Cimbara and Gupta (1965).

5. Conclusions

The experiments carried out show that the 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepine-2-one is reduced at the dropping mercury electrode; the reagent captures two electrons and two hydrogen ions. The only polarographic wave observed is due to a reduction of the azomethine group according to the following scheme:



Evidence of adsorption of the diazepam molecules on the electrode surface is sustained by a study of the electrocapillary curves, and of the current function variation against the scan rate of potential by cyclic voltammetry, and also the oscillopolarographic analysis as a function of the electroactive substance concentration. Further, the reduction wave enables the quantitative determination of the drug in the range of $2{\cdot}5 \times 10^{-6} - 10^{-3}$ M both in acid and basic media.

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