

Investigation of Diazepam by Pulsed Rotation Voltammetry

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Abstract □ Diazepam was investigated in aqueous buffer media using pulsed rotation voltammetry. The dependence of half-wave potentials on pH indicated a two proton involvement in a two electron transfer reduction in the pH range 3~10. Diazepam at micromolar concentration levels may be determined by measurement of the limiting difference current.

Keywords □ Pulsed rotation voltammetry—Glassy carbon rotated disk electrode —Diazepam in aqueous solution— Two electron transfer reduction of the 4,5-azomethine group.

Diazepam, a major anticonvulsant drug, belongs to the class of 1,4-benzodiazepine compounds and shows electrochemical activity at the 4,5-azomethine functional group^{1,2}. Its structure is shown in Figure 1. Since the 1,4-benzodiazepines have extremely low solubilities in aqueous solution (<50 μ M at room temperature), most conventional polarographic studies have employed organic solvents³, organic solvent-aqueous buffer

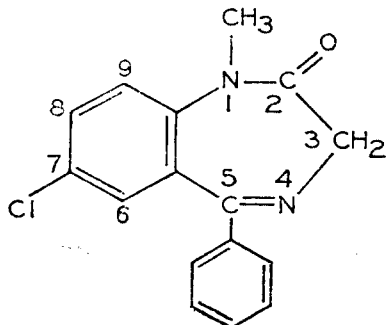
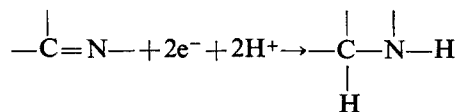


Fig. 1: Structure of diazepam.

mixtures^{1,4,5}, strong acids^{2,6}, or dissolution in strong alkaline solution, immediately followed by diluting with buffer⁷. Strong acid media tend to result in hydrolysis of benzodiazepines⁶. Adsorption of benzodiazepines was reported at higher concentrations than 10⁻⁴ M in sulfuric acid⁶ and sodium hydroxide-phosphate buffer media⁷.

Two protons and two electrons have been postulated for the reduction of the azomethine group¹.



The effect of pH on the diazepam reduction was studied in the pH range 1.2 to 5.6 and $\Delta E_{1/2}/\Delta \text{pH}$ was found to be 55 mV¹, in agreement with this equation.

Recently the pulsed rotation voltammetric technique was used for the studies of the ferricyanide-ferrocyanide system⁸ and of riboflavin, 5-deazariboflavin and flavin coenzymes⁹. Pulsed rotation voltammetry (PRV) involves switching the rotational rate of a working electrode between two values while maintaining a constant potential, so that residual currents due to the surface condition of the solid electrode are compensated. A glassy carbon rotated disk electrode

was employed as the working electrode in the previous studies^{8,9}, since it has a greater cathodic potential range than other solid electrodes. PRV is a sensitive analytical technique for the detection of electroactive species (detection limit at the 10^{-8} M level) and is particularly useful for the electrochemical study of diazepam in aqueous systems.

EXPERIMENTAL

Electrodes and Measurement System

The electrode assembly and measurement system previously reported^{8,9} were also used in the present study.

Reagents

Diazepam powder was of pharmaceutical dosage quality (purity greater than 99%), and was obtained from Hoffmann-La Roche Inc. (Nutley, NJ). All other chemicals were reagent grade and all aqueous solutions were prepared using deionized water (Culligan System). The phosphate buffer solutions contained 0.1 M KCl as a supporting electrolyte and 0.01 M potassium phosphate as a buffer source. Either potassium phosphate monobasic (acidic buffer) or potassium phosphate dibasic (alkaline buffer) was used as the buffer material and the pH of the solutions was adjusted by adding concentrated HCl or 1 N KOH. The biphthalate buffer solutions were prepared from solutions containing 0.1 M KCl and 0.01 M potassium biphthalate, and then either concentrated HCl or 1 N KOH was added to adjust the pH. A 0.01 M KCl solution was used for the reference silver-silver chloride electrode and as a pre-

treating solution.

Procedure

All experiments were carried out at $25.0 \pm 0.5^\circ\text{C}$. The PRV measurement procedure was similar to that described in the previous paper⁹ except for pretreatment. For the following work, the glassy carbon rotated disk working electrode was pretreated by first applying +1.4 V for ten minutes, then -1.8 V for ten minutes, repeating these steps once more and then setting the applied potential to 0 V. A silver-silver chloride electrode in 0.01 M KCl and a spectroscopic graphite electrode were employed as a reference and a counter electrode, respectively. For the PRV measurement, a two electrode system consisting of a glassy carbon rotated disk electrode and a silver-silver chloride electrode was used. The PRV data was analyzed according to the previous procedure^{8,9}.

RESULTS AND DISCUSSION

Effect of pH on Diazepam Reduction

A typical pulsed rotation voltammogram of

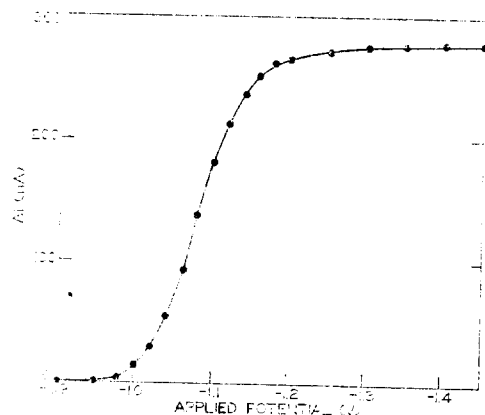


Fig. 2: Pulsed rotation voltammogram of $8.2 \mu\text{M}$ diazepam in pH 6.0 phosphate buffer.

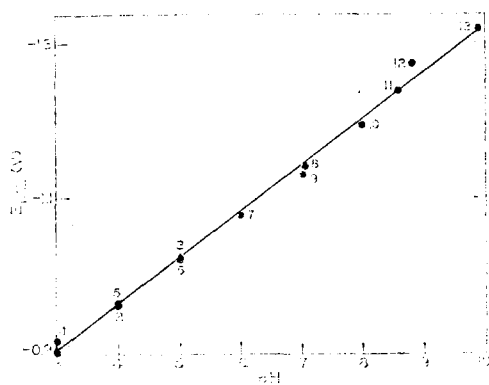


Fig 3: Dependence of halfwave potential on pH for diazepam reduction.

All solutions contain 0.1 M KCl, plus the following buffer components.

Points 1-3: 0.01 M potassium biphthalate, adjusted with conc. HCl or 1 N KOH.

Points 4-8: 0.01 M KH_2PO_4 adjusted with conc. HCl or 1 N KOH.

Points 9-13: 0.01 M K_2HPO_4 adjusted with conc. HCl or 1 N KOH.

diazepam is shown in Figure 2. The well-defined single wave is supportive of a two-electron transfer for the reduction. Similar voltammograms were obtained for twelve other systems in the pH range 3~10. Figure 3 shows the dependence of the half-wave potential on pH. The slope of Figure 3 is 60 mV per pH unit, with a 90% confidence interval of ± 2 mV per pH unit, also supportive of the involve-

Table I: Dependence of limiting difference current upon concentration

Concentration (μM)	$\Delta i_{l,c}^*$ (nA)	$\Delta i_{l,c}/\text{conc.}$ (nA/ μM)
0	15.7 ± 0.5	—
0.158	23.6 ± 0.5	50
0.790	44.7 ± 0.4	36.7
1.58	74.2 ± 0.9	37.0
4.74	196.8 ± 4.1	38.2
9.48	358.0 ± 1.1	36.1

*Average of seven replicate measurements \pm standard deviation.

ment of two protons per two electron transfer. The limiting difference current constant ($\Delta i_{l,c}/\text{conc.}$) was 34.5 nA/ μM , with a standard deviation of 2.6 nA/ μM . This compared very well with a value of 34.9 nA/ μM for the known two-electron reduction of riboflavin on the same electrode⁹.

Concentration Studies of Diazepam

Diazepam solutions were prepared by diluting a 15.8 μM stock solution with pH 4.0 potassium biphthalate buffer. After pretreatment of the working electrode, seven replicate current pulses were measured at an applied potential of -1.200 V (a potential on the plateau) for the buffer solution, and then diazepam solutions in the order of increasing concentration. Results are summarized in Table I. The limiting difference current constant was linearly dependent upon concentration, the median limiting difference current constant ($\Delta i_{l,c}/\text{conc.}$) being 37 nA/ μM .

CONCLUSION

Pulsed rotation voltammetric studies of diazepam in aqueous buffer solutions at the pH range of 3 to 10 showed a single two electron transfer reduction of the 4,5-azome-thine group in the presence of two protons. The present technique may also be used to study other benzodiazepine compounds at micromolar concentration levels.

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LITERATURE CITED

- 1) Senkowski, B.Z., Levin, M.S., Urbigit J.R. and Wollish, E.G., Polarography of some benzodiazepines. *Anal. Chem.* **36**, 1991(1964).
- 2) Berry, D. J., The cathode-ray polarographic determination of diazepam, 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepine-2-one, in human plasma. *Clin. Chim. Acta* **32**, 235(1971).
- 3) Fazzari, F. R. and Riggleman, O. H., Polarographic determination of oxazepam. *J. Pharm. Sci.* **58**, 1530(1969).
- 4) Brooks, M. A., Bel Bruno, J. J., De Silva, J. A. F. and Hackman, M.R., A differential pulse polarographic examination of the 1,4-benzodiazepines. *Anal. Chim. Acta* **74**, 367(1975).
- 5) Goldsmith, J. A., Jenkins, H. A., Grant, J. and Smyth, W. F., The polarographic behavior of the 1,4-benzodiazepines, oxazepam and lorazepam, and a method for quality control. *Anal. Chim. Acta* **66**, 427(1973).
- 6) Jacobsen, E. and Jacobsen, T. V., Electrochemical reduction of chlorodiazepoxide at mercury electrodes. *Anal. Chim. Acta* **55**, 293 (1971).
- 7) Halvorsen, S. and Jacobsen, E., Electroreduction and polarographic determination of nitrazepam in serum. *Anal. Chim. Acta* **59**, 127(1972).
- 8) Blaedel, W. J. and Engstrom, R. C., Investigations of the ferricyanide-ferrocyanide system by pulsed rotation voltammetry. *Anal. Chem.* **50**, 476(1978).
- 9) Blaedel, W.J. and Hahn, Y., Pulsed rotation voltammetry of riboflavin, 5-deazariboflavin, and flavin coenzymes. Submitted to *Anal. Chem.* March (1980).