Analysis of Nitrazepam in Pharmaceutical Dosage Forms by Differential Pulse Polarography

C. SURESH REDDY*, C. SRIDEVI AND S. JAYARAMA REDDY

Dept. of Chemistry, Sri Venkateswara University, TIRUPATI - 517 502.

The differential pulse polarographic behaviour of Nitrazepam has been studied in universal buffers ranging from pH 2.0 to 12.0 in DMF-water mixtures. The optimum pH range for obtaining well resolved peaks for the quantitative determination of Nitrazepam is found to be between 4.0 to 6.0. Both standard addition and calibration methods are used. The lower detection limit is found to be 27 ng mL^{-1}.

SEVERAL methods[1,2] have been reported for the determination of 1,4-benzodiazepines and their metabolites in formulation and biological fluids including high performance liquid chromatography (HPLC)[3]. However much work has been carried out on the determination of nitrazepam in biological fluids[4,5] using only direct current polarography (DCP). The study represent an attempt to develop a simple and rapid differential pulse polarographic method for the analysis of nitrazepam in its pharmaceutical formulations.

EXPERIMENTAL

The differential pulse polarograms were obtained with a metrohm E 506 polarecord connected to E 648 VA-combistand and E 608 VA- controller. A three electrode combination was employed consisting of a dropping mercury electrode (area: 0.0223 cm^2), Ag/AgCl(s), Cl$^-$ electrode and a platinum electrode as an auxiliary electrode. Model LI 120 Elico digital pH meter was used for pH measurements. All the experiments were performed at 25±0.1°C.

Reagents and Solution

Universal buffers of pH 2.0 to 12.0 were prepared by using 0.2M boric acid, 0.05M citric acid and 0.1M trisodium orthophosphate[6]. All the chem-

ical were of analar grade. A stock solution (1x10^{-2}M) of nitrazepam, obtained Sigma Chemical Company, was prepared in dimethyl for mamide (DMF).

Procedure of studying the Polarographic Behaviour

A 0.5 ml volume of the stock solution of nitrazepam was placed in the polarographic cell and 9.5 ml of the appropriate buffer solution of selected pH was added and the solution was purged with oxygen free nitrogen for 15 min prior to each run.

Preparation of Calibration Graphs

Polarograms were recorded for different concentrations of nitrazepam in the concentration range 1x10^{-4}M to 5x10^{-7}M. A graph for measured diffusion current was plotted against nitrazepam concentration. The lower detection limit (dl) was calculated using the expression[7] dl=3sd/m where sd= standard deviation and m = slope of the calibration plot.

Analysis of Pharmaceutical Dosage Forms

Nitrazepam was available in tablet dosage forms. The tablet contains 5 mg of the drug. Ten tablets were weighed and powdered, and the average mass per tablet was determined. A portion of the finely

Table I: Assay of nitrazepam tablets by differential pulse polarography pH =4.0, Pulse Amplitude = 50 mV, Drop Time = 2 sec.

Sample	Labelled amount mg	Amount found mg	Recovery %	Standard deviation
A	5	4.92	98.4	0.021
B	5	4.89	97.8	0.041
C	5	4.99	99.8	0.043
D	5	4.93	98.6	0.016
E	5	4.91	98.2	0.015

ground sample, containing 14 mg of nitrazepam, corresponding to a stock solution of concentration 1×10^{-4} M was accurately weighed and transferred into a 50 ml Calibrated flask containing 40 ml of DMF. The contents of the flask were shaken for 20 min, diluted to the mark with DMF and was filtered. A 1 ml of the clear filtrate and 9 ml of the appropriate buffer of selected pH were placed in the polarographic cell and the polarogram was recorded after deaeration for 15 min with N_2 gas. After obtaining the polarogram, 0.5 ml of the standard solution (0.6 mg/ml) of Nitrazepam was added to the cell,

Deaerated fop 2 min and again polarogram was recorded under similar conditions. The concentration of the unknown nitrazepam solution (C_u) was calculated using the following equation[8]:

$$C_u = \frac{C_s \times V}{V_t \times i_2} \ i_l$$

Where i_l=maximum current of the polarogram of the unknown solution (v), i_2= the maximum current of the polarogram after adding a volume of v ml of the known concentration, C_s = Concentration of the standard solution and V_t = total volume of the solution (V +v).

RESULTS AND DISCUSSION

Nitazepam is found to exhibit two well separated peaks of almost equal heights in the pH range 2.0 to 6.0. The first peak corresponds to the consumption of four electrons in the reduction of nitro group to the hydroxylamine and the second peak is due to the consumption of four electrons in the simultaneous reduction of azamethine ($>C=N-$) group and hydroxylamine. In alkaline solutions also (pH) 8.0-12.0) nitrazepam exhibits a two-step reduction peak. It is assumed that the first peak is due to the identical four electron reduction of the nitro group to hydroxylamine and the second to the two electron azamethine group reduction. In alkaline solutions hydroxylamine is not reduced further owing to insufficient protonation[9]. It is also evident from the fact that in alkaline solution, the second peak is observed to have half then height of first peak.

The diffusion controlled and absorption free nature[10] of the electrode process is evidenced from the linear plots of i_m vs.$t^{2/3}$ passing through origin (where i_m is a a maximum peak current in DPP). The reduction potential (E_p) values of nitrazepam peaks are found to depend on pH and shift towards more negative values with increase in pH of the buffer solutions.
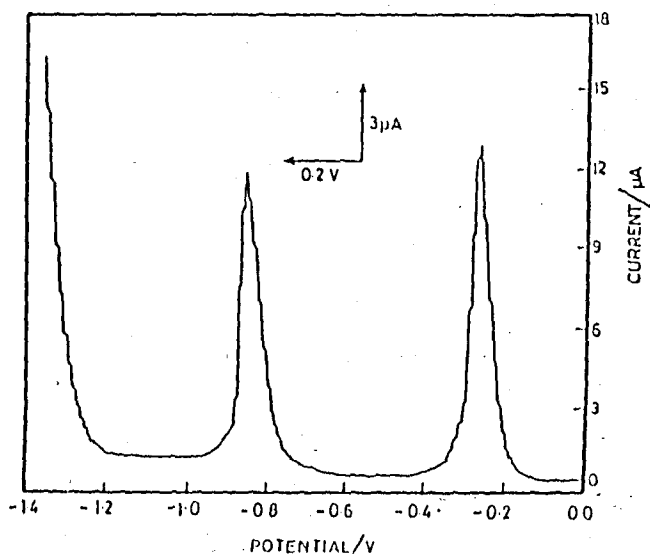
Fig.1. Typical differential pulse polarogram of nitrazepam in pH 4.0
Concentration=0.5m
Drop time = 2 sec

Differential pulse polarography is used for the quantiative estimation of nitrazepam in pharmaceutical formulation using both calibration and standard addition methods. Analysis is carried out using the current obtained for nitro group reduction in nitrazepam **fig.1**. It is observed that at pH 2.0 the nitro group reduction appears at the start to the potential. in alkaline solutions(pH≥8) the reduction of nitro group is not easily facilitated owing to the less available of protons. the optimum pH range for obtaining well resolved peaks for the quantitative determinations of the title compound is found to be pH 4.0 - 6.0. At pH 4.0, the calibration plot of nitrazepam by DPP method is found to be linear in the concentration range 1.5×10^{-5} to 3.2×10^{-7}M with a slope of 0.03 nA ng^{-1} and a detection limit of 27 ng mL-1.

Standard addition method is successfully utilised for the analysis of nitrazepam tablets without any prior separation. The optimum conditions for the analytical determination in pH 4.0 are found to be a drop time of 2 sec and pulse amplitude of 50 m V. The relative standard deviation and correlation co-efficient for (10 replicants) are found to be 1.18% and 0.995 respectively. Table-1 gives the assay result of nitrazepam tablets such as Nitravet (A), Nirven (B), Sedaman (C), Hypnotex (D), and Restorem (E). The recover of 98.2-99.8% obtained with the proposed differential pulse polarographic method indicates its accuracy and reproducibility.

REFERENCES

1. Brooks M.A. and De Silva, J.A.F., **Talanta**, 1975, 22, 849.

2. De Silva, J.A. F. Puglisi, C.V. and Munno, N., J. **Pharm.Sci.**, 1974, 63, 520.

3. De Bruyne, M.M.A. Sinnema, A. and Ver Weiji., **Forensic. Int.**, 1984, 24, 125.

4. Brooks, M.A. and Hackmann, M.J. **Anal. Chem.**, 1975, 47, 2059.

5. Brooks, M. A. Belbruno, J.J. and De Silva, J. A. F., **Anal. Chem. Acta.** 1975,74,367.

6 Perrin, D.D. and Dempsey, B., "Buffers for pH and metal ion control," (Chapman and Hall Publications, London), 1974, p. 156.

7. Smyth, M. R. and Osteryoung J.G. **Aanal. Chem., 1978, 50, 1632.**

8. Hohn, H., "Chemuische Analyser Mitdem Polarographen", (Springer Verlag, Berlin), 1967, p. 51.

9. Suresh Reddy, C. and Jayarama Reddy, S., **Electro-analysis.**, 1992, 4, 598.

10. Smyth, M. R. and Osteryoung, J. G.,**Aanal. Chem. Acta.**, 1978, 96,335.