DETERMINATION OF CLOBAZAM BY DIFFERENTIAL PULSE POLAROGRAPHY

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SUMMARY

The polarographic behaviour of clobazam was investigated over a wide pH range. Different types of peaks were obtained depending on the pH. Characterization of the electroactive process included an examination of the degree of reversibility, electrocapillary curves, influence of surfactant agents and the effect on the peak current of pulse amplitude, ionic strength, drop time, temperature and clobazam concentration. A method for the determination of clobazam, with a detection limit of 4.6×10^{-7} M and a relative standard deviation of 0.97% is proposed.

INTRODUCTION

Clobazam, 1H-1,5-benzodiazepine-2,4(3H,5H)-dione, 7-chloro-1-methyl-5-phenyl is a psychotropic drug which belongs to the 1,5-benzodiazepine group. Several methods have been proposed for its determination(1), most of them are carried out using chromatography (2-10), fluorometry(6)(11) and RIA(12). The use of electroanalytical methods are less frequent; Oelschlager (13) has investigated the polarographic behaviour of several psychotropic agents, among them clobazam, although he also reported on the electroinactivity of this compound. However, Sengun et al.(14), later carried out the determination of clobazam in plasma or serum by polarographic methods (differential pulse polarography, DPP, and cathode ray polarography,CRP) using the reduction peak which this species shows at about -1.5 V in weakly basic media.

The present paper describes a differential pulse polarographic method for the determination of clobazam. The method is accurate, sensitive and easy to apply to routine determination.

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EXPERIMENTAL

Apparatus and conditions for polarographic analysis

A Crison Model 501 pH-meter fitted with a glass-calomel electrode system was employed to measure the pH values of the solutions.

A Metrohm Polarecord E 506 polarograph equipped with a drop timer (Model E 505) was used. A three electrode combination was employed, consisting in an Ag/AgCl reference electrode, a dropping mercury electrode, DME, and a platinum wire as the auxiliary electrode.

A Metrohm EA 880-20 cell was used. Purified nitrogen was bubbled through for 5 min before polarograms were recorded. The instrumental parameters were as follows: applied potential range, +0.2 to -1.8 V., scan rate of 4 mV sec^{-1} and a drop time of 2 sec; pulse amplitude of -20 mV.

Reagents

A 10^{-3} M solution of clobazam was prepared by dissolving 30.0 mg of the compound (Laboratorios Hoechst-Ibérica, Madrid) in 20 ml of methanol (Panreac) and volume was made up to 100 ml with bidistilled water. The solution was always freshly prepared and kept in the dark. Britton-Robinson buffers were prepared with bidistilled water at intervals of 0.5 units over the 2.0-12.0 pH range. Acetate buffer of pH=4.7 was also used.

RESULTS AND DISCUSSION

Effect of pH

Clobazam, in acetate buffer of pH=4.7 exhibits in DPP an oxidation peak at 0.05 V and another reduction peak at -1.35 V. These peaks correspond to the polarographic waves showing Fig.1. The peak currents increased with clobazam concentration and their heights remained constant for at least 48 hours.

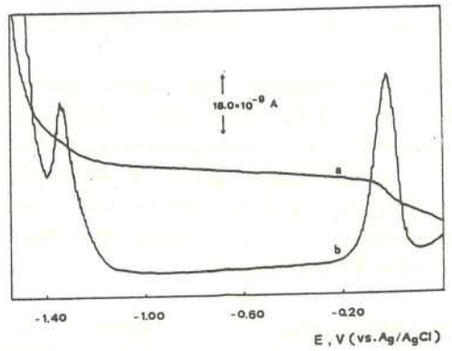

Fig.1. Polarograms of clobazam 1.2×10^{-4} M.
a) Conventional polarography. b) Differential pulse polarography. pH 4.7. Drop time 2 sec.

In Britton-Robinson buffer the oxidation peak appears at 0.05 V, well defined for pH values in the 2-7 range, and the intensity of the peak essentially remains constant in the 3-5 pH range. At pH values over 8, this peak is masked by oxidation of the mercury of the electrode. The reduction peak is observed at pH values above 5 and its intensity reaches maximum and constant values between pH 7 and 11 (Figure 2). The potentials of both peaks remain practically constant, pointing to the non-participation of the H^+ ions in the electrodic process.

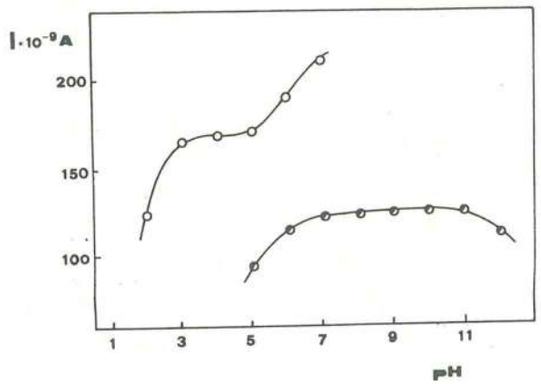

Fig.2. Effect of pH on peak heights.
o Oxidation peak. • Reduction peak. Clobazam 4.0×10^{-4} M. Ionic strength 0.1 M. Drop time 2 sec.

Characteristics of the electrode process

The relationships between peak height and ionic strength, pulse amplitude, drop time, temperature and clobazam concentrations were studied. The influence of ionic strength was studied in clobazam solutions of 2.0×10^{-4} M at pH=4.7 (acetate buffer) by adjusting ionic strength with the buffer itself. It was observed that for both peaks, intensity reached maximum and practically constant values between 0.05 and 0.1 M and decreased for higher concentrations.

The effect of pulse amplitude was studied in the $\pm$ 100 mV range. For values less than $\pm$ 40 mV there is a linear relationship between peak intensity and pulse amplitude, in agreement with the theoretical predictions (15). The influence of temperature was studied between 10-60°C in a 2.0×10^{-4} M solution of clobazam. The peak height/temperature coefficients for both peaks have negative values for high temperatures. Such behaviour suggests the existence of an adsorption process,which are latter confirmed by means of the electrocapillary curves. Furthermore the peak height does not vary linearly with $t^{2/3}$. This behaviour does not agree with the expected values for a diffusion-controlled process.

As may be seen from figure 3, the peak currents exhibited a dependence on the clobazam concentration which resembles a Langmuir adsorption isotherm; a linear relationship was obtained for values lower than 4.0×10^{-4} M.

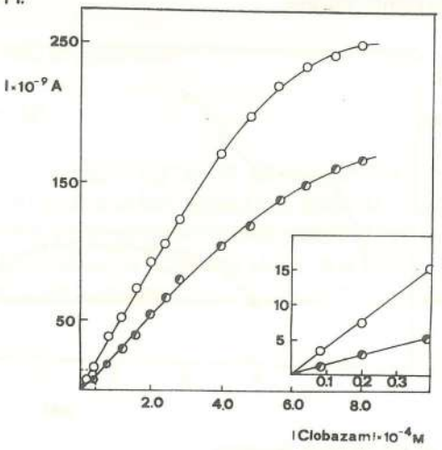

Fig.3. Effect of clobazam concentration on peak heights.
o Oxidation peak. ● Reduction peak. pH 4.7.
Ionic strength 0.1 M.

In order to confirm the existence of adsorption phenomena, the electrocapillary curves for the different species in solution were obtained. The results (Figure 4) show that the curve corresponding to the solutions of clobazam are modified with respect to the background one, with a decrease in the electrocapillary maximum and a shift towards more positive potentials; this seems to confirm the existence of adsorption phenomena onto the surface of the mercury drop electrode.

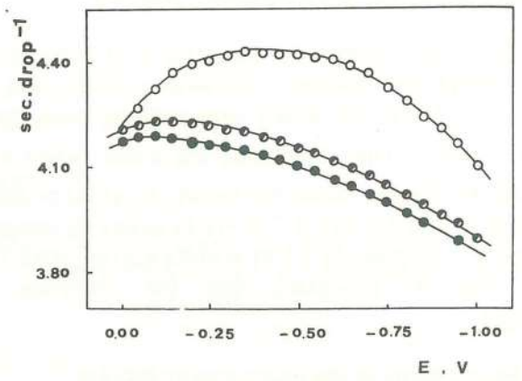

Fig.4. Electrocapillary curves. o acetate buffer 0.1 M (pH 4.7)
● Clobazam 2.0×10^{-4} M, acetate buffer 0.1 M (pH 4.7)
● Clobazam 3.0×10^{-4} M + acetate buffer 0.1 M (pH 4.7)

The influence of surfactant agents was also studied, since it is sometimes possible to obtain information regarding the nature of the species adsorbed onto the electrode. The addition of Triton X-100 causes a decrease in the intensity of both clobazam peaks and almost makes them disappear. When sodium dodecyl sulfate is added, the oxidation peak disappears but the reduction remains unaltered. Finally, in the presence of tetrabutylammonium chloride, the reduction peak disappears but the oxidation remains unaltered. In view of these results, it seems evident that the oxidation peak is due to a negatively charged species, since it is displaced by anionic and non-ionic tensoactives, whereas the reduction peak seems to be originated by a cation because it disappears in the presence of cationic and non-ionic tensoactives.

Moreover, the oxidation peak does not seem to be due to the oxidation of the mercury of the electrode to a mercury-clobazam compound, because when a platinum electrode is used on a solution of 5.0×10^{-4} M clobazam at pH 4.7 ,a peak is observed which is morphologically the same as that obtained with mercury, though displaced about 200 mV towards more positive potentials.

Reversibility of the polarographic process

In order to study reversibility, the criteria of Birke et al (16) were used. These involve the difference between cathodic and anodic peak potential $E_p^c - E_p^a$ and the anodic and cathodic peak currents ratio (i_p^a/i_p^c). The denomination of cathodic and anodic refers to the sign of the impulse applied. Table I shows the results obtained on application of a pulse amplitude of $\pm$20 mV and of $\pm$30 mV to solutions containing various concentrations of clobazam in 0.1 M acetate buffer, pH=4.7. From these results, it may be concluded that the electrode process is quasi-reversible.

Table I. Reversibility of the polarographic process

Clobazam ($\times 10^{-4}$ M)	ΔE (mV) $\pm$	Oxidation peak $E_p^c - E_p^a$ (mV)	Oxidation peak i_p^a/i_p^c	Reduction peak $E_p^c - E_p^a$ (mV)	Reduction peak i_p^a/i_p^c
0.4	20	-12	0.58	-8	0.17
0.8	20	0	0.25	0	0.18
1.2	20	8	0.40	8	0.34
1.6	20	8	0.21	16	0.22
0.4	30	8	0.40	0	0.17
0.8	30	8	0.33	0	0.18
1.2	30	16	0.47	8	0.33
1.6	30	16	0.21	8	0.20

Polarographic determination of clobazam

Both the differential-pulse polarographic peaks may be utilized for the determination of clobazam. However, because the oxidation peak is highly reproducible and it is better resolved at pH 4.7, it was therefore chosen for determination purposes. The peak current varies linearly with the concentration of the drug over the 4.6×10^{-7} M to 4.0×10^{-4} M range. Therefore, using the described cell and instrumental setting, the method is sensitive to about 4.6×10^{-7} M (0.14 µg/ml). This value corresponds to the detection limit, established from the expression: d.l.=3 /m. Upon application of the method to nine solutions of 0.8×10^{-4} M clobazam, a relative standard deviation of 0.97% was found.

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