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VOLTAMMETRIC BEHAVIOUR OF CLONIXIN AND ITS DIFFERENTIAL PULSE POLAROGRAPHIC DETERMINATION IN TABLETS

Key Words: Clonixin, Voltammetry, Differential pulse polarography, Sampled current polarography, Pharmaceutical

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ABSTRACT

Clonixin, a non-steroid analgesic, can be both reduced at the mercury electrode and oxidised at the glassy carbon electrode. The anodic response shows well-defined waves in a pH range between pH 2-12. The polarographic response shows two irreversible waves or peaks in the range between pH 1-6 shifting cathodically when pH increases. Above pH 7.0 all the signals disappeared. The first signal in the dpp mode at pH 1.8 was selected for analytical use. The polarographic

method showed an adequate repeatability, with a CV of 0.7 %. The detection and quantification limits were 2.78×10^{-6} M and 4.67×10^{-6} M, respectively. The dpp technique was applied successfully to both the clonixin uniformity content and composite assay of commercial tablets and compared with a spectrophotometric technique.

Furthermore, pKa calculated by using the 252-nm band pH-dependence was 5.65±0.02, compared with the voltammetric pKa of 6.5.

INTRODUCTION

Clonixin, 2-[(3-chloro-2-methylphenyl)amino]-3-pyridinecarboxylic acid (figure 1), is a non-steroid analgesic widely used in Latin America. It belongs to the fenamate group and although similar to mefenamic acid it is more potent as an analgesic than as an anti-inflammatory, suggesting that clonixin may act on the CNS[1]. Many authors have shown that although there is a strong resemblance between the analgesic effect of clonixin and opiates, clonixin central action is not mediated by opiate receptors[3-6]. Clonixin is very effective in releasing pain produced by nerve compression, renal colic, muscular pain and odontalgia, whereas in pain due to severe trauma involving bone fractures, visceral pain and headaches with a psychogenic component a lower effect was observed[2].

The clonixin pharmacological mechanism of action remains unclear. Analgesia and calcium modulations have been associated in many studies[7]. There are increasing reports that suggest an analgesic effect of calcium channel blockers and with respect to clonixin it is probable that their analgesic activity could be exerted also through a similar action on calcium L-type channels[8].

Clonixin is available commercially as the lysine salt and is administered orally and parentheral. After oral administration clonixin is well absorbed and quickly excreted via renal. Undergoing hepatic metabolism produces hydroxylate metabolites in the 5 position on the pyridine ring and in the 4' and 2' positions of the benzene ring. Such metabolites are excreted within 24 hours in urine[9].

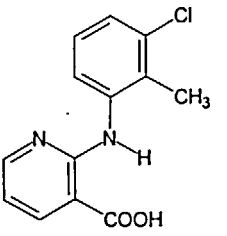


Fig. 1: Chemical structure of clonixin.

The analytical determination of clonixin in plasma has been carried out by using gas chromatography with previous derivatization with diazomethane[10] or N, O-bis(trimethylsililacetamide)[11]. Also, a spectrophotometric method was used to determine drug blood levels[12].

A revision of the literature has given no evidence about electrochemical studies related to clonixin. Consequently, quantitative determination of this drug using electrochemical techniques is a non-explored matter upto today. Considering the great advantage of the electrochemical techniques when applied to drug determination in tablets we have proposed the electrochemical characterisation of clonixin and the development of an electrochemical drug quantification method in pharmaceutical preparations. Also spectrophotometric measurements have been made for comparative purposes.

EXPERIMENTAL

Reagents and drugs

Clonixin as its L-lysine clonixinate salt pure drug (100% chromatographically pure) was obtained from Pharma Investi Laboratory (Santiago-Chile) and commercial tablets of Nefersil® (amount declared 125.0 mg lysine clonixinate per tablet) were commercially obtained. All other reagents employed were of analytical grade and were used without further purification.

Standard solutions

1×10^{-2} M stock solution of clonixin in distilled water was prepared and stored under refrigeration. This solution was stable for at least one month.

Working solutions

For voltammetric studies, dilutions of stock solution were made in supporting electrolyte to obtain concentrations ranging from 5×10^{-5} to 1×10^{-3} M at pH 1.8, or as otherwise stated.

Buffer solutions

For spectrophotometric and voltammetric techniques 0.04 M Britton-Robinson buffer, with ionic strength at 0.3 M with KCl, was used.

Apparatus

Voltammetric and Polarographic System

A Metrohm 693 VA Processor and 694 VA Stand with Ag/AgCl reference electrode (Metrohm), platinum rod auxiliary (Metrohm) and mercury or glassy carbon working electrodes were used. A 20 mL thermoregulated cell (Metrohm) at 20°C was used throughout the experiments. The experimental data were transferred to a 486 PC for evaluation and treatment. The operating conditions were: sensitivity 5 -10 μA; drop time 1 s; potential range 0 to -1700 mV; ΔEp - 5 mV; pulse retard 40 ms; pulse height - 50 mV.

Spectrophotometer

Spectrophotometric measurements were carried out with an UV-Vis spectrophotometer ATI Unicam model UV3, using 1 cm quartz cell and equipped with a 486 computer with Vision acquisition and treatment software.

Calibration curve preparation

A series of ten solutions were prepared containing clonixin concentrations

ranging between 5×10^{-5} and 2.9×10^{-4} M in 0.04M Britton-Robinson buffer at pH 1.8.

Synthetic samples

Excipients (microcristalline cellulose, lactose spray dried, cornstarch, magnesium stearate, lactose, hydroxypropylmethylcellulose, polietileneglicol 4000, titanium oxide and talc) were added to the drug for recovery studies, according to manufacturer's batch formulas for 125.0 mg lysine clonixinate per tablet.

Tablets assay general procedure for uniformity content

Polarography

Ten series of one tablet of Nefersil® (amount declared 125.0 mg lysine clonixinate per tablet) were suspended in 50 mL distilled water. A 1.0 mL aliquot of each solution was taken and diluted to 50 mL with 0.04M Britton-Robinson buffer solution, pH 1.8. Each sample solution was transferred to a polarographic cell, degassed with nitrogen by 5 min. and recorded at least twice from -800 mV to -1200 mV; the mg lysine clonixinate in the sample solution were calculated from a prepared standard calibration curve.

Spectrophotometry

Ten series of one tablet of Nefersil® (amount declared 125.0 mg lysine clonixinate per tablet) were suspended in 50 mL distilled water, sonicated and centrifuged at 3500 rpm. A 1.0 mL aliquot of each solution was taken and diluted to 50 mL with 0.04M Britton-Robinson buffer solution, pH 1.8. Each one sample solution was measured at 328 nm, and the mg of clonixin in the sample solution were calculated from a prepared standard calibration curve.

Tablet Assay

For composite assay 20 tablets of Nefersil® (amount declared 125.0 mg

lysine clonixinate per tablet) were taken and the average weight by tablet was determined. They were pulverized and homogenized in a mortar and a sample of powder equivalent to 125 mg clonixin was weighed and treated as described in *Tablets assay general procedure for uniformity content.*

Apparent pKa determination (pK'a)

For this purpose, the 252 nm UV band was used. The pH solution was changed each 0.5 units and near the pKa zone it was varied each 0.25 units pH. The temperature was kept constant at 25°C and the clonixin concentration used was 5×10^{-5} M for all the pH range.

RESULTS AND DISCUSSION

Clonixin can be both reduced at the mercury electrode and oxidised at the glassy carbon electrode. On the other hand, the lysine moiety did not show electroactivity when comparing with lysine standard solution.

Anodic behaviour

The anodic response was studied with differential pulse voltammetry at the glassy carbon electrode. Well-defined waves were obtained in the pH range between pH 2-12 (figure 2). The dependence of the peak potential (Ep) with pH is shown in figure 3. A cathodic shifting is observed at increasing pH with two zones of different slopes, 59.9 mV/pH between pH 1.5-6.5, and 15.6 mV/pH between 6.5-12. The break around pH 6.5 would indicate a change in the protonation of the electroactive species and may be related to the apparent pKa of the molecule. The peak current *versus* pH curve (inset of fig. 3) resembles a dissociation curve, confirming a voltammetric pKa value around of 6.5.

This oxidation process can be attributed to a pyridine ring oxidation, to produce a N-oxide derivative. Such as oxidation product is in agreement with the

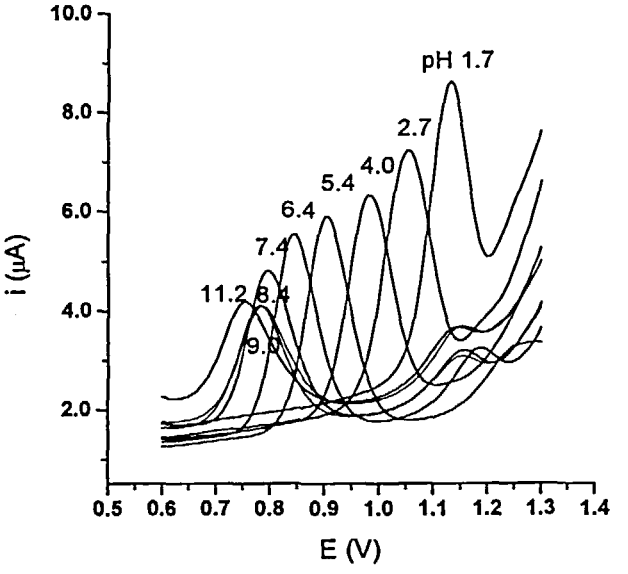


Fig. 2: Differential pulse voltammograms at different pH's of 1×10^{-4} M clonixin solution (Glassy carbon electrode).

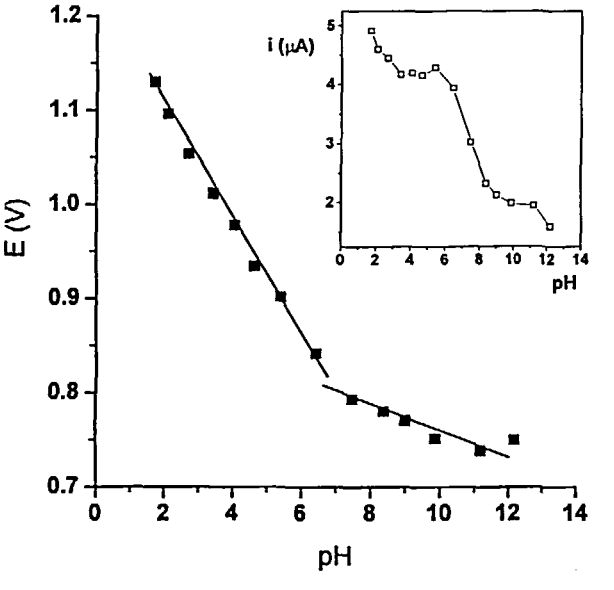


Fig. 3: Potential peak evolution with pH. Inset: peak current evolution with pH. (1×10^{-4} M clonixin solution, Differential pulse voltammetry over glassy carbon

biological N-oxidation of 3-substituted pyridines in which the oxidation of the heteroatom is described as the main metabolic route[13].

In order to prove the dependence of the limiting current with clonixin concentration we have selected pH 5.0. Furthermore, the area of the curve proved to be a more convenient parameter for obtaining the calibration curve (Area, $\mu A \times V$ = -8.364 + 4.562×10^6 C, M) and a linear range was found for clonixin concentrations between 6×10^{-5} M and 1.9×10^{-4} M.

The observed results indicate that the anodic behaviour could be useful for electrochemical detection in an HPLC method.

Cathodic behaviour

The polarographic response by differential pulse polarography (dpp) and sampled current polarography (tast) at different pHs are shown in figures 4 and 5, respectively. Two waves or peaks are observed in the range between pH 1-6, shifting cathodically when pH increases. Above pH 7.0 all the signals disappeared. The first signal appeared approximately at –1.0 V *vs* Ag/AgCl and was used for analytical applications. The second one appeared around –1.3 V, near the supporting electrolyte discharge. Furthermore, the second wave appeared in the tast polarogram with a strong polarographic maximum. This fact is indicative of adsorption of the electroactive species.

In figure 6 the evolution of the peak potentials with pH is shown. For the first wave we can observe two linear zones with a break at approximately 3.5. The second peak presents a linear behaviour with a slope of 72.6 mV/pH.

The evolution of the limiting current with pH is shown in the insert of figure 6. The first wave has a slow current decrease with increasing pH, whereas the second peak height is strongly pH-dependent suggesting a catalytic hydrogen wave. This type of wave is very common in nitrogenated heterocyclic compounds[14, 15].

From the above results we have discarded the second signal for analytical purposes, because the limiting current of this wave was an adsorptive type and

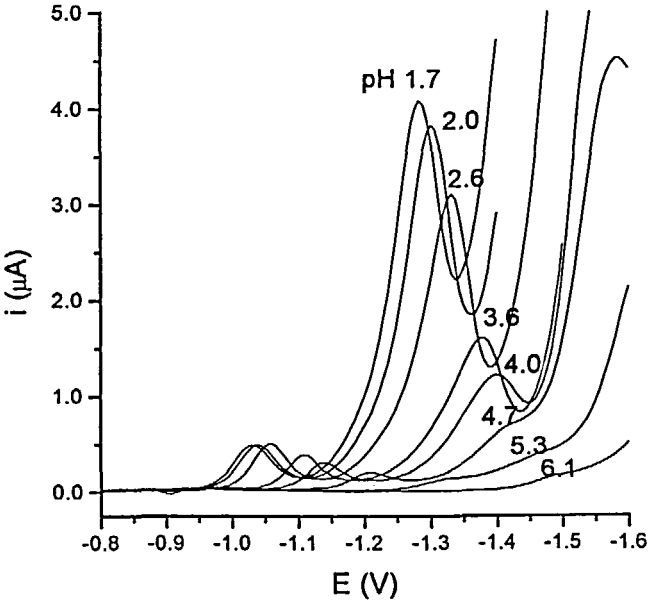


Fig. 4: Differential pulse polarograms at different pH's of 1×10^{-4} M clonixin solutions (DME).

catalytic, without linear dependence on clonixin concentration. Consequently the first signal in the dpp mode at pH 1.8 was selected.

The peak current of this signal shows a limiting current diffusion-controlled as was derived from the following behaviours: a) a temperature coefficient of 0.4% $°C^{-1}$ between 15°C and 40°C was obtained; b) a linear relation between the clonixin concentration and the limiting current.

Similar behaviour has been previously founded for nicotinic acid and nicotinamide, in which the π electrons of the pyridine ring increases the interaction with the mercury surface[16].

On the other hand, cyclic voltammetric experiments show only one irreversible cathodic peak up to scan rates of 5 V/s for clonixin solutions. The peak

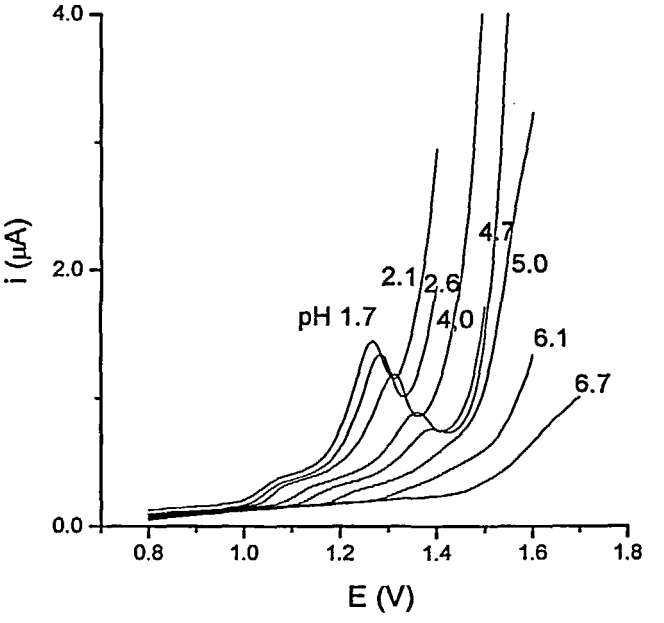
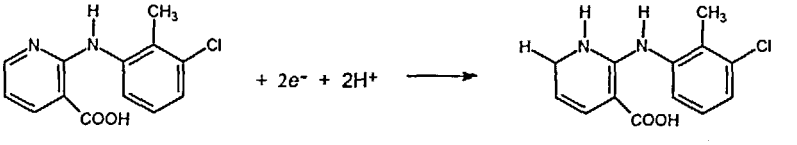


Fig. 5: Sampled current polarograms at different pH of 1×10^{-4} M clonixin solutions (DME).

current shows a linear relationship with the square root of the scan rate indicating that the electrochemical process is controlled by the diffusion of the electroactive species to the electrode surface.

The following overall scheme was proposed for the reduction occurring at the pyridine ring, this moiety being easier to be reduced to generate the 1,2-dihydropyridine derivative.



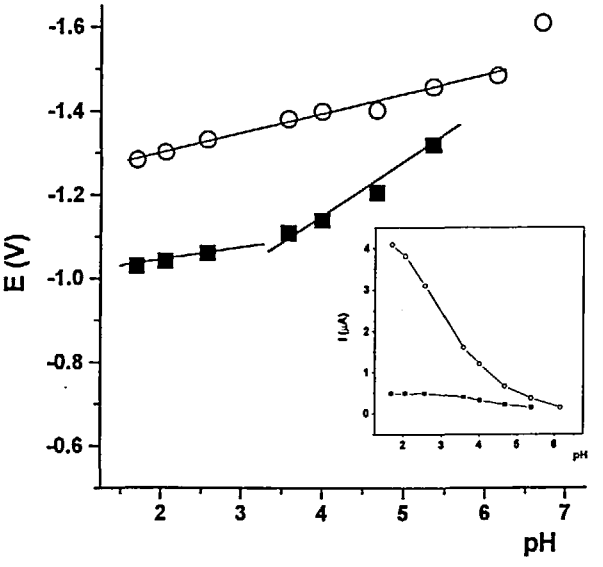


Fig. 6: Potential peak evolution with pH. Inset: peak current evolution with pH. (1×10^{-4} M clonixin solution, Differential pulse polarography on DME). (○: first peak; ■: second peak)

The clonixin molecule is structurally related to the fenamates and also to the nicotinic acid. For the latter and related derivatives, the presence of two polarographic waves at similar potentials to that for clonixin have been reported[17,18].

UV Spectroscopy

Lysine clonixinate solutions exhibit three absorption bands at 252, 284 and 328 nm. In figure 7, the behaviour of the UV-spectra with pH is shown. From these spectra, it appears that only the 252 and 284 nm bands are pH-dependent, with an isosbestic point at 265 nm. From this pH dependence, the spectrophotometric pKa was calculated by using the 252-nm band pH-dependence; a value of 5.65±0.02 is

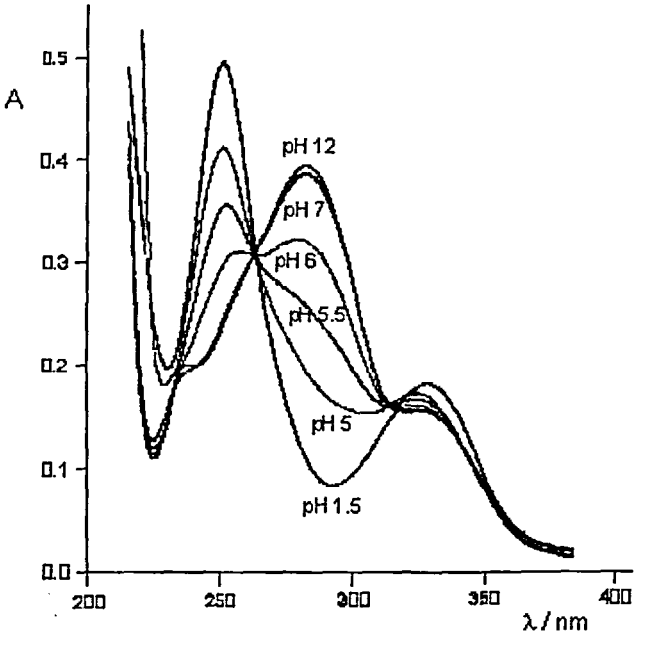


Fig. 7: UV-spectra of 1×10^{-5} M clonixin solution with pH.

related to the previous voltammetric pKa of 6.5 (typically the voltammetric pKa differs from the spectrophotometric one by ± 1 unit). This pKa value can be attributed to the carboxyl moiety of the 3-position of the pyridine ring.

Analytical applications

The polarographic response has been applied for the quantitative assay of clonixin in pure drug and in dosage forms. For the validation of the analytical procedure, linearity, repeatability and recovery tests were performed.

Based on the greater resolution and sensitivity, a working pH of 1.8 and the dpp technique were selected for quantification. The polarographic method showed an adequate repeatability, with a CV of 0.7%. The detection and quantitative limits were 2.78×10^{-6} M and 4.67×10^{-6} M, respectively.

TABLE 1. Recovery of Clonixinate* from Synthetic Samples by Differential Pulse Polarography and UV-Spectrophotometry

Sample	Differential pulse polarography	UV-spectrophotometry
1	98.10	99.68
2	96.68	99.32
3	98.19	99.62
4	99.08	99.55
5	98.50	98.68
6	98.05	98.27
7	97.75	98.70
8	97.89	99.23
9	98.85	99.66
10	98.60	99.39
Average	98.17	99.21
sd	0.67	0.49
CV, %	0.69	0.50

*Synthetic mix prepared containing 125 mg of clonixin standard and excipients

For assay the calibration plot method was used, obtained from nine points between 5.0×10^{-5} M and 2.6×10^{-4} M expressed by the following equation:

$$Ip\ [\mu A] = 5105.044\times C\ [M] - 0.032 \qquad (r= 0.9997, n= 9),$$

where Ip is the peak current, C is the clonixin concentrations, r is the regression coefficient.

In Table 1, the recovery study is shown and indicates that both polarographic and spectrophotometric techniques are adequately precise and accuracy, with CV's lower than 1% and percentage of recoveries near 100.0%. It is recommended for the determination of clonixin in tablets.

TABLE 2. Individual Tablet Assay for Nefersil Tablets (Declared Amount 125 mg/Tablet)

Sample	dpp	UV
1	126.2	127.1
2	124.6	126.6
3	125.3	126.8
4	123.2	126.6
5	125.6	127.7
6	128.7	130.7
7	123.4	127.0
8	129.2	131.7
9	122.9	129.9
10	125.8	126.4
Average	124.5	128.1
sd	4.39	1.95
CV, %	3.52	1.52

The dpp technique was applied to the clonixin uniformity content of commercial tablets (Table 2), and compared with the spectrophotometric technique. The results show that both techniques have similar distribution but the dpp method exhibits a greater standard deviation than UV.

The dpp developed method was applied successfully to the composite assay of 125.0 mg lysine clonixinate in commercial tablet formulations, obtaining an average found of 122.5 mg (percentage found over declared = 98.0%), with a CV of 2.7 %, that is in accord with the general USP limits for AINES formulated in tablets (± 10%)[18].

Based on all the above results, we consider that the developed method is a good tool to be applied to clonixin determination in pharmaceutical products, with adequate reproducibility and recovery. Furthermore, the polarographic analysis was not time consuming and the excipients did not interfere in the analysis, avoiding separation steps.

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