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Short communication

Polarographic behaviour of Aceclofenac, Tenoxicam and Droxicam in a methanol–water mixture

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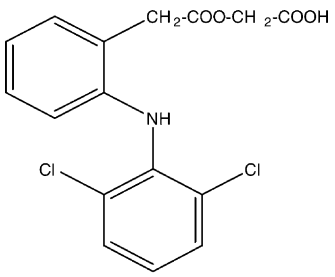
Abstract

A polarographic study about how three anti-inflammatories, such as Aceclofenac, Tenoxicam and Droxicam behave, using tast polarography (TP) and differential pulse polarography (DPP) was carried out. These studies were always carried out in a media formed by Methanol–Britton–Robinson aqueous buffer (0.1 M) (4:96 (v/v)) due to the low solubility of these drugs in water. A strong influence of pH on analytical signals was observed, showing that the optimal pH values were between 4 and 5. Using DPP in the optimal experimental conditions, a detection limit of 10 ppb for Tenoxicam and Droxicam and 52 ppb for Aceclofenac was reached. The DPP proposed method was successfully applied to the determination of the active compounds in commercial drugs.

Keywords: Aceclofenac; Tenoxicam; Droxicam; Tast polarography; Differential pulse polarography

1. Introduction

Aceclofenac, or 2-{(2,6-dichlorophenyl)amino}-phenylacetoxyacetic acid, is a non-steroidian anti-inflammatory agent of relatively recent appearance in the European pharmacopeia. This active principle corresponds to the following formula:

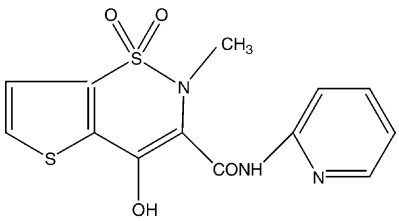


ACECLOFENAC

Its properties as active principle have been previously described by Vire et al. [1].

Tenoxicam and Droxicam are non-steroidian anti-inflammatory drugs of the oxicam family that are carboxamic *N*-heterocycles derived from benzothiazin-1,2-dioxide. These drugs have anti-inflammatory and analgesic properties, mainly by prostaglandin inhibition as well as by leukocyte migration and phagocyte inhibition [1]. Due to their hydrophobic properties, these molecules are quite insoluble in water but soluble enough in organic and hydro-organic media [1] as well as in micellar media [2].

The pharmacological and metabolic behaviour of Tenoxicam, or 4-hydroxy-2-methyl-*N*-2-pyridil-2*H*-triene(2,3-*e*)-1,2-thiazin-3-carboxamide 1,1-dioxide, has been studied by Nilsen et al. [3]. A comparative study with respect to aspirin [4], was carried out by Bird et al. The efficacy, the mechanisms and the action of the drug as well as its applications have also been described [5].



TENOXICAM

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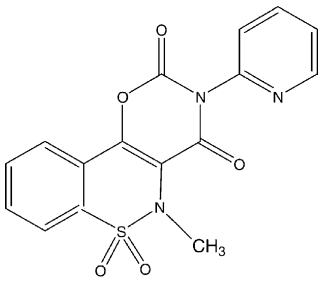
Studies on its determination in human plasma by HPLC have been described [6–8]. With respect to its separation, identification and determination, a full study on human urine samples of patients and volunteers after oral administration of Tenoxicam, was carried out by Dell et al. [9], using HPLC.

Droxicam, 5-methyl-3-(2-pyridil)-2*H*,5*H*-oxazino(5,6-*c*)-(1,2)benzothiazin-4,4(3*H*)-dione-6,6-dioxide, is shown below.

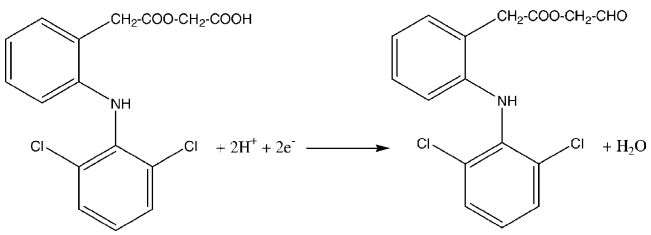


DROXICAM

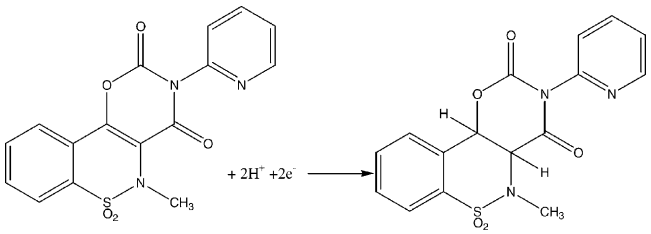
This drug is an anti-inflammatory compound [10], of remarkable gastro-intestinal tolerance [11] and powerful activity.

The pharmacokinetics and the metabolism of Droxicam have been described by Farré et al. [11] and by Esteve et al. [12].

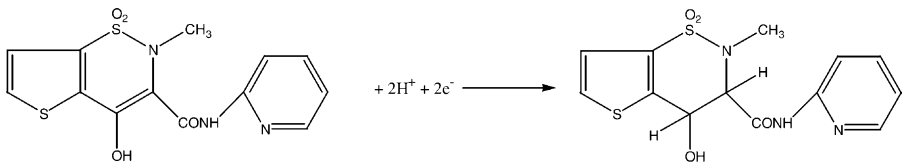
With respect to the electrochemical reactions of the two drugs studied, Aceclofenac is reduced on mercury electrode according to the following way:



In the case of Droxicam, the electrochemical reduction is the following:



And finally, the electron exchange in Tenoxicam, was due to the double bond as shown below:



As can be noticed, the electrochemical reductions of Droxicam and Tenoxicam were similar.

As the three drugs are reducted on mercury, we have proposed to study them by electroanalytical techniques. These techniques, in general, can be applied after a simple preparation of the sample and provide good detection limits.

Here, these compounds were studied by using two polarographic techniques: (1) tast polarography (TP); (2) differential pulse polarography (DPP).

2. Experimental

2.1. Reagents

Aceclofenac, Tenoxicam and Droxicam were purchased from Prodesfarma, Roche and Pfeifer, respectively.

Methanol and all other reagents were obtained from Merck and were of Analytical Grade.

A methanolic 1×10^{-3} mol/l stock solution of each anti-inflammatory (except for Aceclofenac, 5×10^{-3} mol/l) was prepared by dissolving an adequate amount of the pure product in methanol up to 25 ml. A Britton–Robinson buffer aqueous solution containing each component acid at 0.1 mol/l was used as the supporting electrolyte. The pH value required was obtained by adding 0.2 mol/l NaOH solution. All measures were carry out in a media consisted of: 4% methanol–96% B–R solution (v/v).

The commercial drugs analysed, were: Falcol® (100 mg/pill of Aceclofenac); Ombolam® (20 mg/capsule of Droxicam) and Tilcotil® of (20 mg/pill of Tenoxicam) from Bayer, Dr. Esteve S.A and Roche Laboratories, respectively.

2.2. Apparatus

The polarograms were obtained with a Metrohm E 506 Polarograph with a Metrohm 648 Polarographic Poste. Metrohm cells 1415.210 and EA876-20 (double wall), were used.

Electrodes: an Ag/AgCl/KCl as reference, a platinum wire as counter and a Metrohm 6.1230.010 capillary as working were used.

A Crison micro-pH 2002, a Tamsom TC Thermostat and a Selecta Ultrasonic unit were also utilised.

2.3. Polarographic procedure

2.3.1. General procedure

In order to know the shape of the electrochemical signals and to propose an electroanalytical determination procedure

Table 1
Optimum operational selected for the determination of drugs solutions using both polarographic techniques

Parameters	Variation interval	Optimum values		
		Aceclofenac	Tenoxicam	Droxicam
pH	2–14	4	4	5
Scan rate (mV/s)	2–15	4	3	7.5
t_{drop} (s)	0.4–1.2	1	1	0.4
T (°C)	20–60	25	25	25
Pulse amplitude (mV)	−(10–100)	−100	−50	−50

for the three studied drugs, 5×10^{-5} M solution of Aceclofenac and 2.5×10^{-5} M for Tenoxicam and Droxicam, were separately measured by using TP and DPP. Methanol buffer aqueous solutions (4:96 (v/v)) were employed and before each measurement, oxygen free nitrogen was bubbled through the solutions for 15 min to deareate them. Measurements were made at room temperature with the following initial conditions: scan rate of 4 mV/s, t_{drop} of 1 s. and $\Delta E = -50$ mV; the ionic strength was the one provided by the B–R buffer.

The influence of several parameters on the polarographic signal, such as pH, potential scan rate, t_{drop}, temperature, pulse amplitude (DPP) and analyte concentration (only on DPP), were studied. The operational parameters and their optimum value selected using both techniques are showed in Table 1.

2.3.2. Drug formulation control (by DPP)

The content of five pills (or capsules) of each commercial preparation was well ground and mixed in an agate mortar. An accurate amount of this solid mixture for each drug was dissolved in pure methanol in a 25 ml volumetric flask in order to obtain about a 1.0×10^{-3} M drug sample solution. Then, each solution was filtered in order to eliminate the excipients. Therefore, six working solutions of each product were prepared introducing: (A) 0.75 ml of pure methanol plus 0.25 ml of Aceclofenac sample solution or (B) 1 ml of pure methanol plus 50 μl of each other drug samples in a volumetric flask of 25 ml and diluted with Britton–Robinson buffer at the adequate pH value. The obtained solutions were about 10^{-5} M to Aceclofenac and 2×10^{-6} M to the other two.

Each of these solutions was measured and after several standard additions of 50 μl of 5×10^{-3} M Aceclofenac solution or 25 μl of a 10^{-3} M other drugs solutions were done in order to determine the accurate contents of anti-inflammatory in the commercial drugs.

3. Results and discussion

3.1. Aceclofenac

When the influence of pH in Aceclofenac reduction intensity was studied by TP and DPP, a similar behaviour was observed in both cases: intensity increased with pH did up to a value of four, after that it decreased up to pH 6 and from this value it remained constant (Fig. 1a). Moreover, in both techniques the reduction potential became more negative with the pH up to a value of pH 4 where it started being toward more positive values between pH 4 and 6 and then remained constant for the rest of pH values (Fig. 1b). Because of these results, pH 4 was selected as the optimum value for further experiments. Here, DPP was not more sensitive than TP.

The effect of temperature on wave and peak current for an Aceclofenac solution using TP and DPP at pH 4 was studied and the temperature coefficients (τ) were calculated according to Meites [13] obtaining a coefficient of approximately 2% by TP and random values by DPP, which confirms a diffusional and an adsorptive component, respectively, in the reductive electrode process. So, 25 °C was chosen as the optimal temperature value.

The effect of pulse amplitude on intensity and peak potential was also studied on DPP (Fig. 2) and as can be observed, the ΔE influence on peak intensity was linear, affecting positively the sensitivity as well as the detection and determination limits of these electroanalytical methods for this drug. However, in the case of potential peaks no change was observed. In addition, the half-peak width tended to be constant when the amplitude was changed, which confirms the adsorptive component in the reductive process already observed in the study of temperature influence. Consequently, $\Delta E = -100$ mV was selected as the optimal value of the pulse amplitude for further experiments.

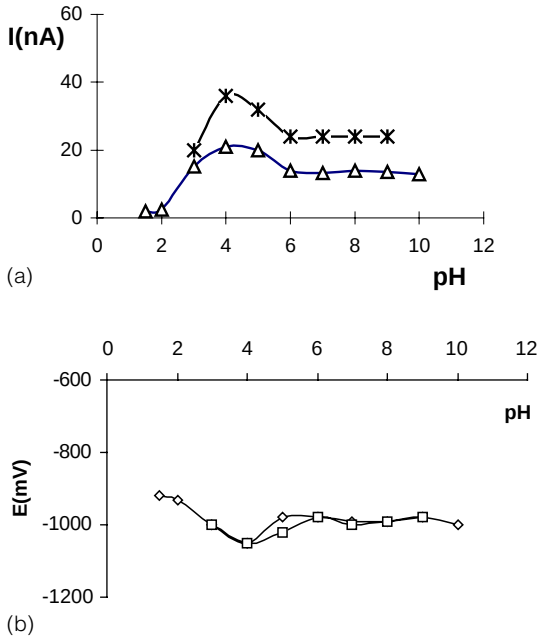


Fig. 1. Influence of pH on: (a) I_{lim} (✱) and I_p (△); (b) $E_{1/2}$ (□) and E_p (◇) of Aceclofenac 5×10^{-5} M obtained by using TP and DPP in Methanol–Britton–Robinson buffer, 0.1 M (4:96 (v/v)), 4 mV/s and t_{drop} 1.0 s, $\Delta E = -50$ mV.

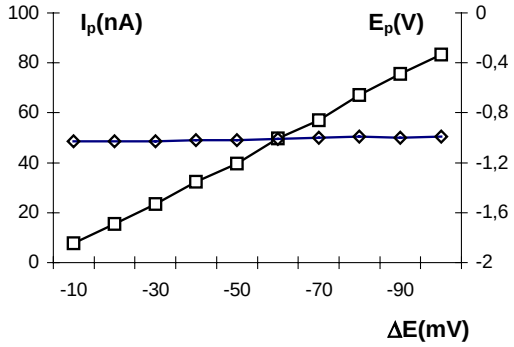


Fig. 2. Influence of ΔE on I_p (□) and E_p (◇) for Aceclofenac 5 × 10^{-5} M obtained by using DPP in the same conditions as in Fig. 1.

The calibration graph in the optimal experimental conditions was obtained in a concentration range between 1.0 × 10^{-6} M and 1.0 × 10^{-4} M with the following equation:

$$I_p\,(\text{nA}) = 2.03 + 2.15\,\text{C}\,(\text{M} \times 10^{-6}); \qquad r^2 = 0.990$$

The determination and detection limits were 4.88 × 10^{-7} M (172 ppb) and 1.47 × 10^{-7} M (52 ppb), respectively.

Finally, the electrochemical behaviour of Aceclofenac was studied by means of TP in a solution at pH 4, by applying Tome's [14] ($E = E_{1/2} + (0.059/n)\log(I/I_d - I)$) and Meites's [13] [$(E_{1/4} - E_{3/4}) = (0.0564/n)$ V] criteria, obtaining, respectively, a straight line with 0.130 of slope and $\Delta E = 0.190$ V. These values were different from the theoretical expected values for a reversible process that exchanges two electrons (slope = 0.028 and $\Delta E = 0.0282$ V), so it can be concluded that the electrochemical reduction of Aceclofenac was irreversible in these experimental conditions.

3.2. Tenoxicam

When the pH influence in TP for this compound was studied, a first wave appeared from very acid pH values, whose intensity remained almost constant up to pH 4.5 and then the intensity decreased. A second and weaker wave appeared at −1.3 V (SCE) from pH 5.8, whose height increased slightly, with a maximum at a pH near to neutrality (Fig. 3a). In the same figure, half-wave potentials versus pH are plotted. As can be seen, the potential of both half-waves was displaced toward negative values with pH.

On the other hand, in DPP, peak intensity and peak potential versus pH are shown in Fig. 3b. In all the range of pH values studied, two peaks always appeared, the first one much more sensitive than the second. Whereas, the intensity of the first peak increased with the pH up to a value of pH 4 and then decreased, the intensity of the second one remained virtually constant. Moreover, the potential of the two peaks (Fig. 3b) become more negative with the pH, as happened with the half-wave potential in TP. These facts can be interpreted in the sense that there is a clear intervention of protons in the electrochemical reduction. For all pH values DPP was more sensitive than TP.

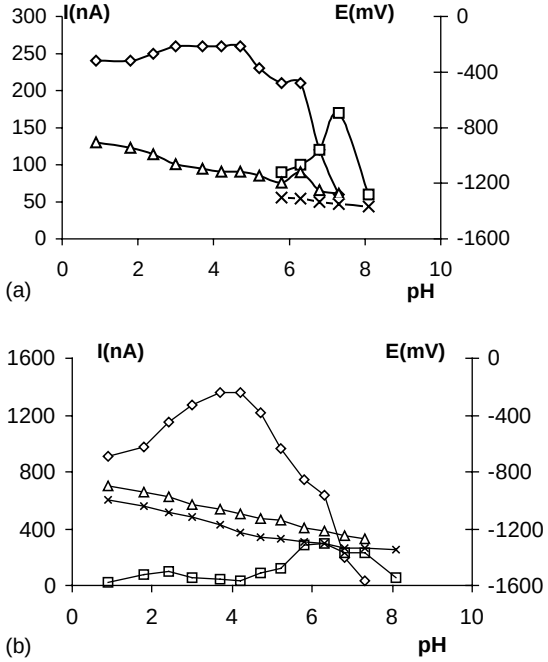


Fig. 3. Influence of pH on: (a) $I_{\text{lim}(1)}$ (◇), $I_{\text{lim}(2)}$ (□) and $E_{1/2(1)}$ (△), $E_{1/2(2)}$ (×); (b) $I_{p(1)}$ (◇), $I_{p(2)}$ (□) and $E_{p(1)}$ (△), $E_{p(2)}$ (×) of Tenoxicam 5 × 10^{-5} M obtained by using TP and DPP in Methanol–Britton–Robinson buffer, 0.1 M(4:96 (v/v)), 3 mV/s and t_{drop} 1.0 s, $\Delta E = -50$ mV.

For quantitative determination pH 4, as optimum value for the following experiments and the height of first peak on DPP, was chosen.

In Fig. 4a and b, some polarograms showing the effect of pH obtained by TP and DPP, respectively, are shown.

The effect of temperature on the reduction of Tenoxicam was studied by both techniques. In TP, we can say the same for the first wave as with the one regarding to Aceclofenac, but concerning the second one, the temperature coefficients were somewhat superior to 2%. Therefore, we cannot determine with precision, the limiting current nature (probably

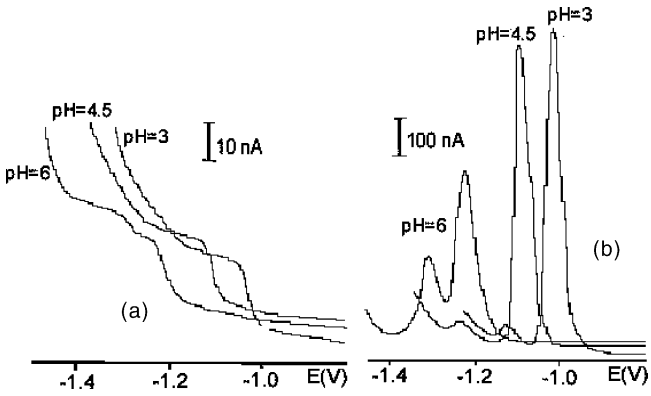


Fig. 4. TP and DPP of a Tenoxicam 5 × 10^{-5} M solution at different pH values in Methanol–Britton–Robinson buffer, 0.1 M(4:96 (v/v)); scan rate: 3 mV/s, $t_{\text{drop}} = 1.0$ s $\Delta E = -50$ mV.

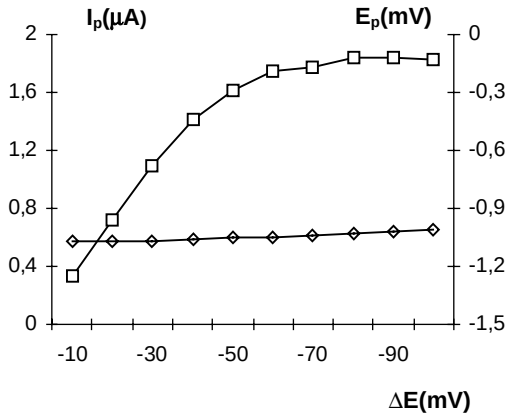


Fig. 5. Influence of ΔE on I_p (□) and E_p (◇) for Tenoxicam 5×10^{-5} M obtained by using DPP in the same conditions as in Fig. 3.

mixed adsorption and diffusion process). The effect of temperature using DPP was the same as that described for Aceclofenac with the same technique. Thus, 25 °C was chosen as the optimal temperature value.

In DPP, the influence of ΔE on I_p and E_p (only for the first peak) is plotted in Fig. 5. A linear influence on I_p, between 0 and −50 mV, was obtained. Over this last value, intensity remained practically constant and as can be seen in Fig. 5 a slow modification in the peak potential also observed. A $\Delta E = -50$ mV value was chosen as the optimal for further experiments.

The calibration graph in the optimal experimental conditions was obtained in a concentration range between 1.0×10^{-7} M and 1.0×10^{-5} M with the following equation:

$$I_p\,(\text{nA}) = 3.05 \times 10^{-1} + 26.51\,\text{C}\,(\text{M} \times 10^{-6});$$
$$r^2 = 0.9998$$

The determination and detection limits were 1.0×10^{-7} M (34.0 ppb) and 3.0×10^{-8} M (10.2 ppb), respectively.

Finally, the electrochemical behaviour of Tenoxicam was studied in a solution at pH 4 (only for the first wave), by means of TP and applying Tome's [14] and Meites' [13] criteria, obtaining a straight line with 0.023 of slope and $\Delta E = 0.021$ V, respectively. These values were similar from the theoretical expected values for a reversible process that exchange two electrons (slope = 0.028 and $\Delta E = 0.0282$ V), so, it can be concluded that the electrochemical reductions of Tenoxicam was reversible in these experimental conditions.

3.3. Droxicam

Over the studied pH range, for both the techniques, one reduction signal appeared with the same evolution with pH (Fig. 6a), increasing up to pH 5 and then decreasing and disappearing at pH 9. For all pH values, DPP was more sensitive than TP.

Regarding to the signal potential, it became more negative with an increase of pH in both techniques, as can be seen

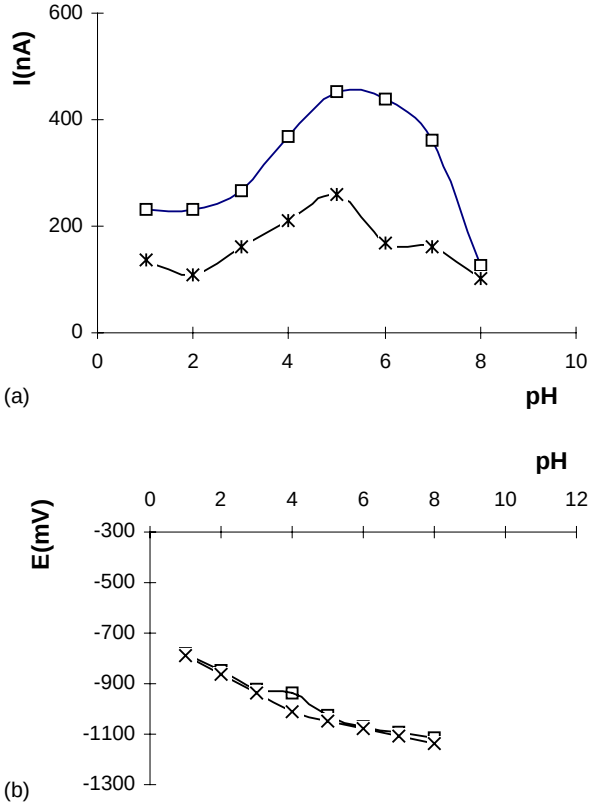


Fig. 6. Influence of pH on: (a) I_{lim}(✱) and I_p(□); (b) $E_{1/2}$ (□) and E_p (×) of Droxicam 5×10^{-5} M obtained by using TP and DPP in Methanol–Britton–Robinson buffer, 0.1 M (4:96 (v/v)), 7.5 mV/s and t_{drop} 0.4 s. $\Delta E = -50$ mV.

in Fig. 6b and pH 5 was selected as the optimum for the following experiments.

The effect of temperature on the reduction of Droxicam was studied with both techniques. In TP, we can affirm that this drug does not undergo any catalytic or kinetic process because, except for 25 °C, the temperature coefficients are less than 2% (1.47% for 30 °C and 1.10% for 55 °C), indicating that this drug is adsorbed on the electrode or decomposed by the heat. By DPP, we can observe that the peak current increases up to 45 °C and then the intensity decreases because a second peak appears due to the decomposition of Droxicam (partial hydrolysis with transformation in Piroxicam [15]). So, 25 °C was chosen as the optimal temperature value.

The electrochemical behaviour of Droxicam was studied at pH 5 in the optimal conditions by means of TP, applying Tome's [14] and Meites' [13] criteria, obtaining, respectively, a straight line with a slope = 0.082 and $\Delta E = 0.200$ V. These values were different from the theoretical expected values and it can be concluded that the electrochemical reduction of Droxicam was irreversible in these experimental conditions.

Regarding to the DPP the effect of pulse amplitude on the intensity and peak potential was also studied. The obtained results are shown in Fig. 7, in which an exponential

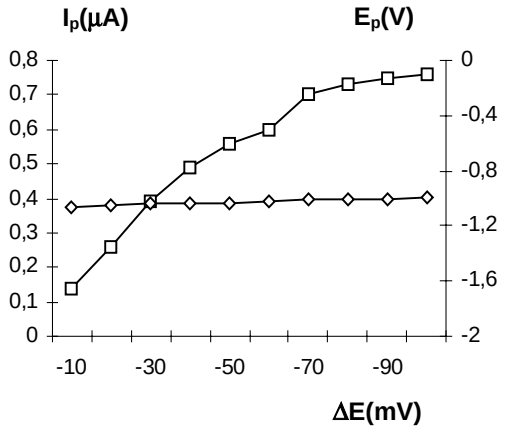


Fig. 7. Influence of ΔE on I_p (□) and E_p (◇) for Droxicam 5×10^{-5} M obtained by using DPP in the same conditions as in Fig. 6.

variation of the function $I_p = f(\Delta E)$ and a slight decrease of the function $E_p = f(\Delta E)$, are observed. This different behaviour with respect to the other drugs is probably due to its instability in solution and its transformation to Piroxicam. In conclusion, $\Delta E = -50$ mV was selected as the optimal value of the pulse amplitude for further experiments.

The calibration graph in the optimal experimental conditions was obtained in a concentration range between 1.0×10^{-7} M and 1.0×10^{-5} M with the following equation:

$$I_p\,(\text{nA}) = 5.37 + 23.58\,\text{C}\,(\text{M} \times 10^{-6}); \qquad r^2 = 0.9995$$

The determination and detection limits were, 9.3×10^{-8} M (33.2 ppb) and 2.8×10^{-8} M (10.0 ppb), respectively.

After the study of the electrochemical behaviour of these anti-inflammatories by using TP and DPP, we have proved that the most suitable method for their quantitative determination is DPP, due to its good reproducibility and low detection limit. Because of that, we propose the DPP as a technique to determine these drugs in different pharmaceutical compositions.

3.4. Drugs assay in formulations

In order to know the accurate content of commercial drugs, based, respectively, in Aceclofenac, Tenoxicam and Droxicam, pills of falcol, ombolan and tilcotil, were analysed by using the proposed DPP method. The standard additions method was chosen for this study so the background effects could be minimised. The mean results obtained for each drug determinations($n = 6$) are showed in the Table 2, in good agreement with the declared values from the manufacturer.

Table 2
Determination of Aceclofenac, Droxicam and Tenoxicam in real samples using DPP

Sample	mg of drug/pill (manufacturer)	mg of drug/pill found[a]
Falcol (Aceclofenac)	100	100 ± 3
Ombolam (Droxicam)	20	21 ± 2
Tilcotil (Tenoxicam)	20	20 ± 2

[a] Mean value of six determinations.

4. Conclusions

According to the obtained results, a very easy, sensitive and rapid method is provided for these drugs by DPP, with a good detection limits although, in the case of Aceclofenac, this is less sensitive than its determination by DPV on Carbon paste electrode [16]). In addition the measurements do not require any complex sample preparation.

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