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Contribution to the Electrochemical Behavior Study of Piroxicam in Different Aqueous-Organic Media and Electrodes by Using Polarographic and Voltammetric Techniques

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ABSTRACT

A study of the electrochemical behavior of piroxicam has been carried out by dc polarography, classical and differential pulse mode voltammetric techniques, as well as by classical and differential pulse mode adsorptive cathodic stripping voltammetry (AdCSV), in methanol/H_2O(20/80% v/v) and acetonitrile/H_2O(20/80% v/v) media. Several parameters, such as pH, temperature, scan rate, ad-accumulation potential, and accumulation time, elucidate the piroxicam reduction mechanism and establish analytical methods, mainly by AdCSV, obtaining a detection limit of 2.4×10^{-11} M.

KEY WORDS: Piroxicam, Voltammetric determination, Aqueous-organic media, Hanging mercury drop electrode.

INTRODUCTION

Piroxicam is a new antiinflammatory drug of the oxicam family that is composed of carboxamidic N-heterocycles derived from benzothiacin-1,2-dioxyde-1,1. Piroxicam acts as an antiinflammatory mainly by prostaglandin synthesis inhibition, as well as by leucocyte migration and phagocyte activity inhibition [1].

The piroxicam molecule is relatively water insoluble due to its hydrophobic character. In contrast, it is readily soluble in organic, aqueous-organic, and micellar media [1,2].

According to Viré et al. [1], piroxicam is polarographically reducible, giving a well-defined wave. These authors gave an excellent explanation of its reduction process by using electrochemical and optical observations [1,6]. They confirmed that piroxicam is also electrochemically oxidizable on several carbon electrodes [4–6].

The identification of reduction products was carried out by Kauffmann et al. [6] on a mercury pool in acidic and alkaline media by using quantitative electrolytic techniques. According to these authors, the process takes place by the irreversible reduction of the double bond of the enol function over the entire pH range studied. Nevertheless, in alkaline and acidic media, a preceding two-electron step occurs, producing opening of the thiacin ring. In the pH 4 to 7 region, the electrochemical reduction showed only one step, corresponding to the reduction of the enol function double bond. However, between pH 2 and 4, the same authors confirm that the acidity is too poor to produce the breaking of the carbon nitrogen bond, and a mixture of products is found [6].

Some piroxicam methods have been reported in the literature; spectrophotometry was carried out by Sastry and Rao [7] and by Hu and Zhou [8], with a detection limit of 3.7×10^{-6} M and 6.04×10^{-5} M. Many high-performance liquid chromatography (HPLC) methods of this drug have been performed [9,10], and these methods were applied to the analysis of samples, such as urine, plasma, and drugs [11], by using several extracting solvents [10,12–14], several mobile phases [15,16], and several stationary phases and columns [10,16]. The detection was carried out mainly by spectrophotometry at several wavelengths or by fluorimetry at 400 nm. The detection limit was between 9.05×10^{-10} and 1.51×10^{-7} M. Gas chromatography has also been employed for piroxicam determination, utilizing a flame ionization detector [17].

Viré et al. [1] proposed a piroxicam polarographic method with a detection limit of 5×10^{-8} M and a voltammetric method, by utilizing a carbon paste electrode,

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whose detection limit was 5×10^{-7} M. El-Maali et al. [18] have published a very interesting article in which a comparison between piroxicam and tenoxicam has been carried out at the hanging mercury drop electrode (HMDE) using square-wave sripping voltammetry and obtaining detection limits of 7×10^{-10} M.

The aim of this article is to clarify the nature of the interaction between piroxicam and the mercury electrode by using DC polarography and classical and differential pulse voltammetry. We have differentiated the diffusion phenomenon from the adsorption one [19] mainly by studying the influence of temperature, piroxicam concentration, etc., on reduction voltammetric peak height. Moreover, we have carried out several experiments on the piroxicam reduction mechanism in order to complete, as far as possible, the explanation of the quantitative electrochemical reactions of this drug [6].

EXPERIMENTAL

Apparatus

Voltammetric peaks were obtained by using the following instruments: an Amel 551 potentiostat, Amel 566 function generator, Amel 560 interface unit, and Linseis LY 17100 recorder, as well as an Amel system formed by a potentiostat-galvanostat 553, an Amel 568 function generator, a Philips PM 8271 X-Y-t recorder, and a Metrohm E 506 polarograph. A Metrohm EA 290 HMDE as working electrode was used, as well as a drop mercury electrode (DME). As reference electrode, a Ag/AgCl, KCl saturated electrode was utilized.

To control the temperature and pH, a Tamson TC 3 thermostat and a Crison 501 digital pH-meter were utilized, respectively. To weigh the solid reagents and to measure small volumes, a Sartorius analytical balance and a Pipetman micropipette were employed, respectively.

Reagents

The piroxicam used was kindly provided by Pfizer (Spain). All of the inorganic reagents (NaOH, HCl, KNO_3, NaH_2PO_4, NH_4Cl, NH_4OH, etc.), utilized to prepare the solutions were "pure reagents." The water utilized was deionized by using a Barnstead system (nanopure grade). Organic solvents, such as methanol and acetonitrile, were also "pure reagents" furnished by Merck.

Procedure

Since piroxicam is sparingly soluble in water, a mixture of methanol/H_2O(20/80% v/v) or acetonitrile/H_2O(20/80% v/v) was used as solvents. The composition of the supporting electrolyte was fixed by adding the required amount of 0.5 M KNO_3 solution, the required pH buffer, the organic solvent, as well as the deionized water required to make a 25 ml volume.

A preliminary electrochemical study of piroxicam in a methanol media, utilizing AC polarography as well as differential pulse polarography, had very poor results when E_p versus pH was plotted. Moreover, the results obtained in the same media by Viré et al. [1] by plotting $E_{1/2}$ versus pH were difficult to interpret. For these reasons, we have utilized acetonitrile in order to compare the piroxicam electrochemical behavior in both solvents.

The experimental parameters studied were temperature, pH, scan rate, analyte concentration, ad-accumulation potential, ad-accumulation time, etc., on peak potential, peak height, half-wave potential, etc.

RESULTS AND DISCUSSION

Several aspects will be considered: (1) voltammetric study of piroxicam in methanol/H_2O(20/80% v/v) media and (2) voltammetric study of piroxicam in acetonitrile/H_2O(20/80% v/v) media. In addition, by using different piroxicam concentrations assays in which the main process was diffusion controlled and assays in which the main process was adsorption controlled were carried out.

Voltammetric Study of Piroxicam in Methanol/H_2O

Diffusion-Controlled Process. To study the electrochemical behavior of piroxicam in order to know the true reduction mechanism and to establish sensitive and selective analytical methods by using voltammetric techniques, a 4×10^{-5} M solution of piroxicam was prepared.

The scan rate influence has been studied between 20 and 100 mV/s.

By measuring the peak heights and plotting their values versus $v^{1/2}$ and after adjusting the experimental values by the least-square method, a straight line was obtained, whose equation is the following:

$$i_p = -1.25 + 0.365\, v^{1/2}$$

where i_p denotes the peak heights in microamps and v denotes the scan rates in millivolts per second. The correlation coefficient was 0.9999.

Taking into account these results, the Randles-Sevcik equation for the reduction voltammetric peak of piroxicam was followed. Nevertheless, on carrying out several cyclic scans, a peak decrease was observed at all of the assayed scan rates, as has been reported (1).

The scan rate influence on the peak potential has also been studied in acidic media (pH 1.0 and 4.7). At pH 1.0 and 4.7, no variation of this parameter was observed, obtaining in all cases a potential of −960 mV (Ag/AgCl, KCl sat.). Apparently, the piroxicam behaves electrochemically, in these conditions, as it is not clearly irreversible, although one does not observe any oxidation peak at the reverse scan, according to Viré et al. (1). These experiments confirm that the electrochemical behavior of piroxicam is not very clear, at least in some respects.

pH Influence. With regard to the pH influence, it was observed that the best results (i.e., high and sharp peaks) were in acid media, between pH 2 and 5.

Adsorption Process on the Electrode. In order to know whether an adsorption process of piroxicam on

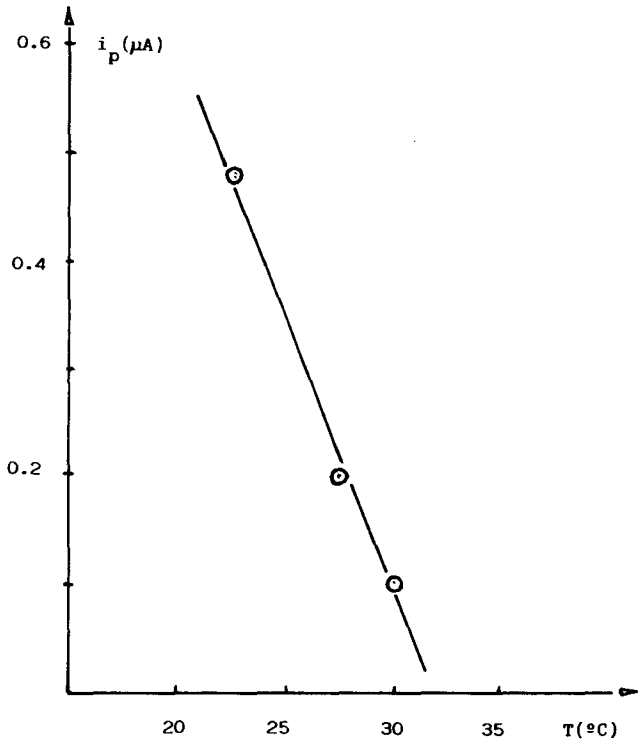

FIGURE 1. Influence of temperature on voltammetric peak heights of 5×10^{-7} M piroxicam in methanol/H_2O(20%) in acid media on a HMDE.

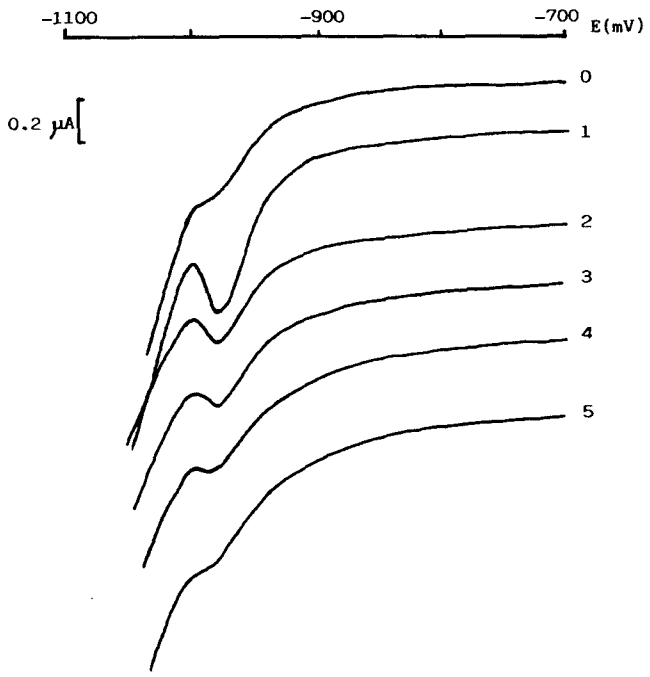

FIGURE 2. Influence of ad-accumulation time on voltammetric peak heights of 5×10^{-7} M in methanol/H_2O(20%), in acid media on a HMDE at 100 mV/s: (**0**) 0 min; (**1**) 1 min; (**2**) 2 min; (**3**) 3 min; (**4**) 4 min; and (**5**) 5 min.

the HMDE can really take place, a 5×10^{-7} M piroxicam solution at pH 1.85 was prepared.

Temperature influence. No signal was observed in the direct linear scan mode. By applying an accumulation potential of −600 mV (Ag/AgCl, KCl sat.), however, responses were obtained at ad-accumulation times of 0 and 1 minute for 20.5, 25.5, and 30.0°C.

Results have shown that the higher the temperature, the lower are the peaks. In Figure 1, the influence of the temperature on the peak heights is plotted. From this figure it was deduced that an adsorption process of piroxicam undoubtedly takes place on a mercury electrode.

Effect of Ad-Accumulation Time. As shown in Figure 2, the peak height decreases for ad-accumulation time longer than 1 minute due probably to a blocking of the electrode surface by the piroxicam reduction products or by electrode surface saturation by the piroxicam itself.

Scan Rate Influence. These experiments with a 10^{-7} M piroxicam solution were carried out at pH 1.0 and pH 4.7. The scan rate values were between 10 and 200 mV/s, and in all cases, the peak potential remained constant at −1080 mV, indicating that, at least in these conditions, the piroxicam reduction appears as reversible, because according to Laviron(20), when an oxidized substance is adsorbed on an HMDE and is electrochemically reduced, the peak potential variation with the scan rate, for irreversible electrochemical systems, follows the equation

$$E_p = E^\circ + RT/\alpha n_a F \ln (RT/\alpha n_a F k/v)$$

where v is the scan rate and the other terms are well known.

On the other hand, the scan rate influence on peak height of adsorbed piroxicam was studied and it was found that i_p varies linearly with v and by plotting i_p versus v (at pH 1.0, as well as at pH 4.7) straight lines were obtained with equations

$$(\text{pH } 1.0)\ i_p = -1.45 + 0.78\,v \quad r = 0.9988$$

$$(\text{pH } 4.7)\ i_p = 0.23 + 0.60\,v \quad r = 0.9987$$

where i_p denotes the peak height in nanoamps and v the scan rates in millivolts per second.

These equations do not indicate that the adsorbed piroxicam behaves reversibly, because according to Laviron(20), these same equations could be followed by both reversible and irreversible processes on a mercury electrode. However, if the oxidation peak in the reverse scan is not observed, it could also be due to the formation of an electroinactive product, and not only to the irreversible character of the piroxicam. There are many organic substances that reduce irreversibly in a diffusion process and reversibly in an adsorption process (e.g., alkaloids, other organic acids, the anodic oxidation of car-

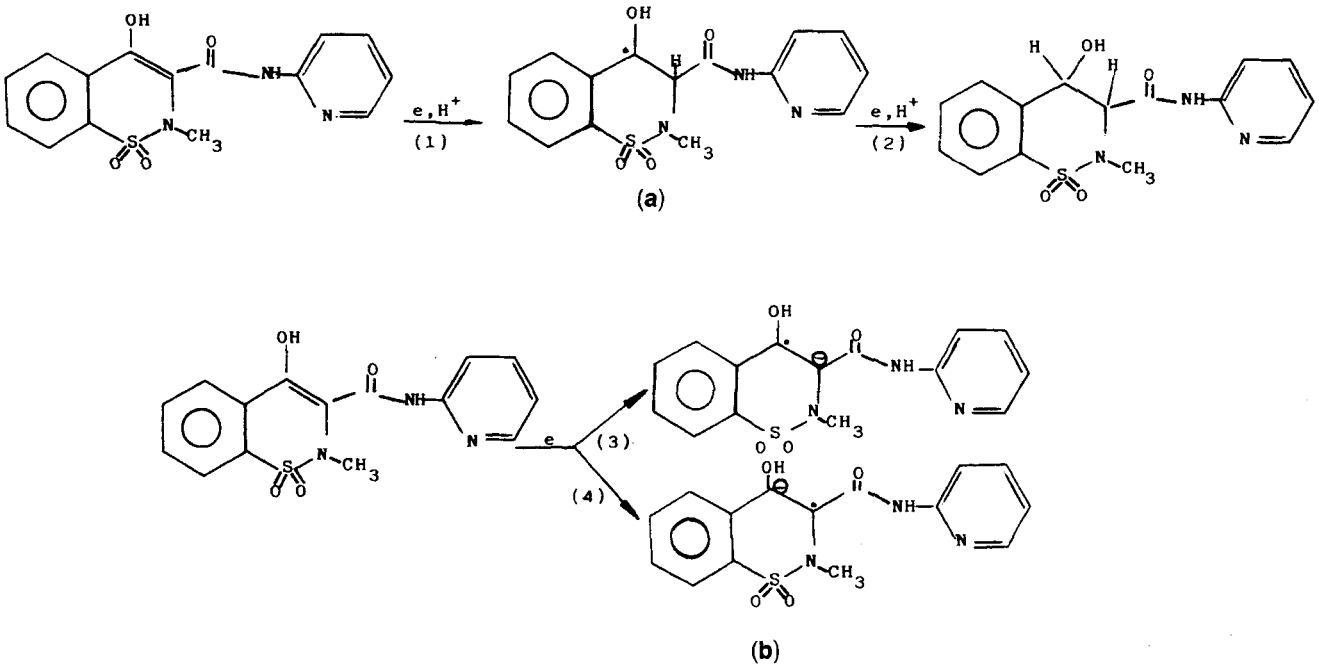

FIGURE 3. (**a**) Proposed mechanism for the electrochemical reduction of piroxicam in medium HAcO/AcO^- (pH 4.7) on mercury electrode; (**b**) probable ways for the first step of piroxicam electrochemical reduction in weakly acid media (pH 4.7).

boxylates, etc. [21]). Nevertheless, these equations were used to calculate the r^* values; that is, the piroxicam surface concentration (adsorbed piroxicam mass on the mercury electrode) in mol/cm^2 at $t = 0$ was very high(1), and the lower the pH, the more intense was the adsorption process.

Voltammetric Study in Acetonitrile/H_2O

Diffusion-Controlled Process. Solutions of piroxicam were prepared to have a concentration of 10^{-4} M; therefore the diffusion process will be predominant.

In order to compare our results with those obtained by other authors (1), a preliminary study was carried out using DC polarography. A series of polarograms with a 4×10^{-4} M piroxicam solution (with the same conditions as in the Experimental section) at pH between 0.97 and 11.78 were obtained that were the same as those obtained by Viré et al. (1), although not all of the waves described by these authors were obtained. In fact, above pH 8.29, only a small wave at about −1450 mV potential was seen.

On plotting the function $E_{1/2} = f(\text{pH})$, between pH 0.97 and 8.29, a straight line (with only one slope) followed the equation

$$E_{1/2} = -978.83 - 53.41 \text{ pH}$$

with a correlation coefficient of 0.984.

Moreover, a logarithmic study of all of the polarograms recorded between pH 0.97 and 8.29 gave a straight line (for pH 0.97) whose equation was the following:

$$\log (i_d - i)/i = 15.77 + 0.0154E$$

with a correlation coefficient of 0.993. By measuring the slope of this straight line, a value of 64.9 mV/log unit was obtained, showing that polarographically the piroxicam behaves irreversibly. Thus, if the piroxicam reduction is irreversible, the polarographic wave equation is, according to Bond(22), the following:

$$E = E^{\circ\prime} - 0.059/\beta n_a \cdot m\text{pH} + 0.059/n_a \log (i_d - i)/i$$

where $E^{\circ\prime}$ is $E + 0.059/\beta n_a \log 0.886 k_s (t/D)^{1/2}$, E the normal potential, β the symmetry factor (remember that the symmetry factor coincides with the charge-transfer coefficient when the electrochemical process takes place in only one monoelectronic step), n_a the electron number exchanged in the rate-determining step (rds), k_s the charge-transfer rate constant, t the drop time, and D the diffusion coefficient of piroxicam.

On comparing the equations $E_{1/2}$ and E, one can write the following:

$$0.059\text{m}/\alpha n_a = 0.053$$

where α is the charge-transfer coefficient, n_a the exchanged electron number in the rds, and m the exchanged proton number, and taking $n_a = 1$, one obtains for the m/α ratio a value at about 1, over the all of the pH range studied. Thus, if one considers that the exchanged proton number is 1, the charge-transfer coefficient could be 1.

With regard to the piroxicam reduction mechanism, according to Bockris and Reddy (23), all of the transfers involving several electrons are made according to the tunnel effect theory, in which only one electron is in-

volved in every step. Therefore, if one considers the electrochemical reduction of piroxicam in acidic media, both a four-electron transfer and two-electron transfer are carried out by steps, one of which is called the rate-determining step (rds). So, taking this into account and a constant slope on plotting $E_{1/2} = f(\text{pH})$, a bielectronic process in HAcO/AcO$^-$ medium (pH 4.7) is proposed. The piroxicam reduction mechanism is given in Figure 3. In this figure, one can also see two possible ways for the first step, with step 3 the most probable because this canonical form is stabilized by resonance.

In this figure, step 1 is very rapid, so on the contrary, step 2 must be very slow; that is, this step must be the rds. According to Bockris and Reddy (23), the charge-transfer coefficient α is given by the equation

$$(\alpha = \gamma/\nu + r_\beta)$$

where γ is the preceeding step number, ν the stoichiometric coefficient, r the electron number exchanged in the rds, and β the symmetry factor. Thus, if one assumes that γ is 1, that ν is 2, that r is 1 and that β is 0.5, one can conclude that the charge-transfer coefficient must be 1, a conclusion in accord with the experimental results.

In the case of a very acidic media (pH 1), according to Viré et al. (1), piroxicam, following a tetraelectronic process, is reduced in two steps, the first transferring two electrons and two protons and breaking the thiacin ring and the second transferring two electrons and two protons by reducing the double bond of the enol function. However, because the double bond reduction is the only electrochemical reaction occurring in a weakly acidic media (pH 4.7), the easiest is the double bond reduction. Therefore, in strong acidic media, the first step will be the double bond reduction and the second one will be the molecule reduction with the breaking of the thiacin ring. Nevertheless, the overall result is the same.

In order to optimize the piroxicam electroanalytical method, several parameters, such as pH, temperature, scan rate, drop size, and analyte concentration, were explored.

pH Influence. All measurements were made in acetonitrile /H_2O(20% v/v) adjusted at pH 4.7 (HAcO/AcO$^-$). The higher the pH, the lower the $-E_p$. By plotting E_p versus pH (Figure 4) and by the square-least method, the following equation was obtained:

$$E_p = -925.8 - 67.5\ \text{pH}$$

whose correlation coefficient was 0.9978.

Temperature Influence. On varying the temperature from 20°C to 40°C, a peak height increase of 20.7% per °C was observed. Therefore, the process is not of an adsorptive nature.

Drop Size Influence. In order to improve the measurements, the electrode drop size was modified utilizing an HMDE EA 290 Metrohm and a 10^{-4} M solution of piroxicam. From the plotted data, an adjusted straight line whose equation was as the following was obtained:

$$i_p = 53 + 72n$$

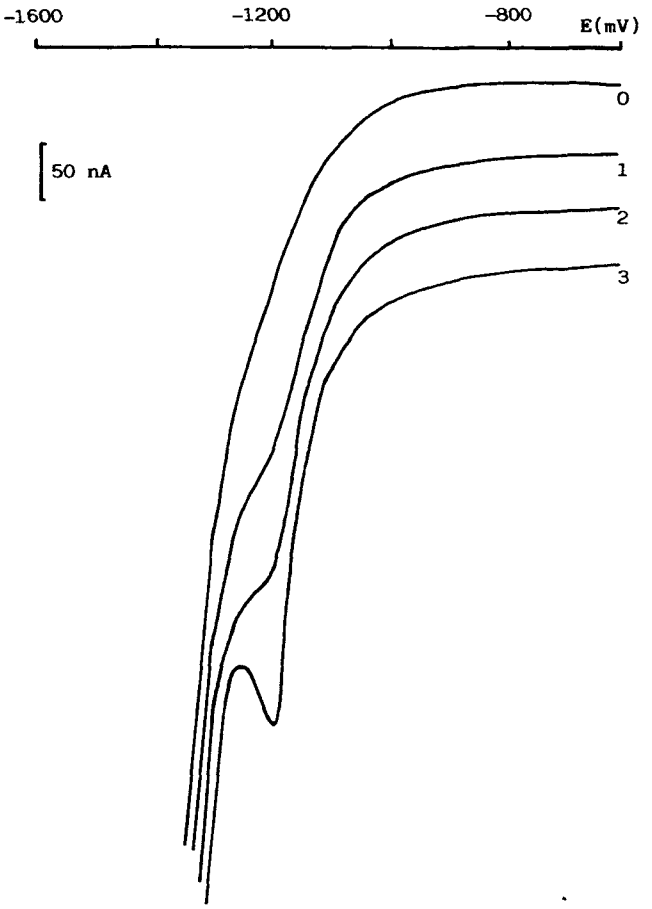

FIGURE 4. Influence of piroxicam concentration on voltammetric peak heights in acetonitrile/H_2O(20%) on an HMDE; scan rate, 100 mV/s; pH, 4.7.

where i_p denotes the peak heights in microamps and n the division numbers of the micrometer screw, with a correlation coefficient of 0.999. Good linearity up to the fifth division was obtained. In all the experiments utilized three divisions corresponding to a surface of 1.82 0.04 mm^2.

Scan Rate Influence. With a piroxicam solution prepared as above, the scan rate influence on the peak height between 10 and 200 mV/, scanning the potential between −600 and −1500 mV (Ag/AgCl, KCl sat.) was studied. By plotting i_p versus v, the following equation was obtained:

$$i_p = 17.08 + 1.30v$$

with a correlation coefficient of 0.988, showing that the equation of Randles-Sevcik is not exactly followed.

Analyte Concentration Influence. Several solutions of piroxicam, whose concentrations were between 9.95×10^{-7} M and 6.04×10^{-5} M, were prepared. A linear response range was obtained with the following equation:

$$i_p = 0.21 + 3.09C$$

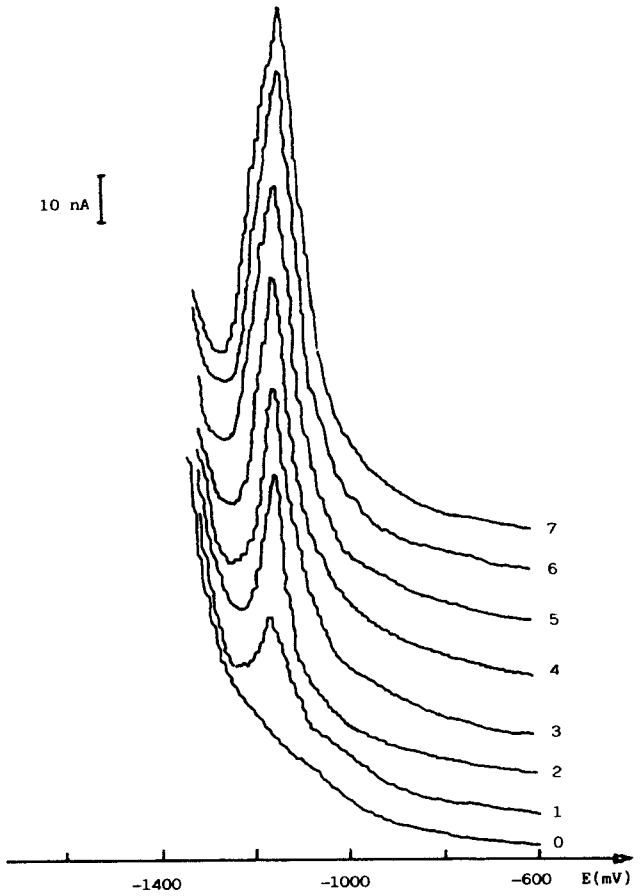

FIGURE 5. Influence of piroxicam concentration on voltammetric peak heights (differential pulse mode) in acetonitrile/H_2O(20%); pH, 4.7; scan rate, 25 mV/s.

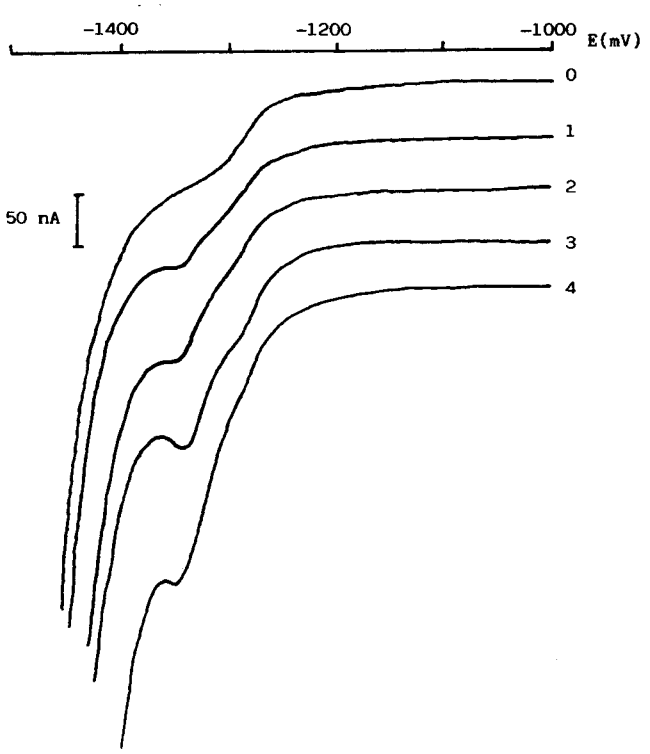

FIGURE 6. Influence of ad-accumulation time on voltammetric peak heights of 6×10^{-8} M piroxicam on HMDE; scan rate, 100 mV/s; (**0**) supporting electrolyte; (**1**) 0 s; (**2**) 15 s; (**3**) 30 s; and (**4**) 45 s.

where i_p denotes the peak height in microamps and C the piroxicam concentration in mols per liter, and whose correlation coefficient was 0.9997.

In order to determine the detection limit, several solutions were prepared whose piroxicam concentrations were between 2×10^{-7} and 2.8×10^{-7} M (Figure 4).

From these values and after plotting the calibration graph adjusted by the least-square method, a detection limit of 2.45×10^{-8} M was obtained (according to the IUPAC, that is, $C_{o1} = 3Sy/x/p$), where S is the standard deviation and p the calibration graph slope.

In order to improve the obtained results, experiments by differential pulse voltammetry were carried out at an HDME and utilizing a $\Delta E = -50$ mV and a scan rate of 25 mV/s. The solution utilized was prepared as follows: 5 ml of 0.5 M KNO_3, 5 ml of 0.5 M HAcO/AcO^- (pH 4.57), 5 ml of acetonitrile and deionized H_2O to make 25 ml.

To this solution several volumes of 15 μl of 1.05×10^{-4} M piroxicam solution were added, and the voltammetric peaks, by utilizing three divisions of the micrometer screw, were recorded and plotted in Figure 5.

By plotting i_p versus piroxicam concentration, a straight line was obtained whose equation was the following:

$$i_p = 4.53 + 1.78 \times 10^{-8} C$$

where i_p denotes the peak heights in nanoamps and C the piroxicam concentration in mols per liter. The correlation coefficient was 0.9987 and the detection limit $2.74 \cdot 10^{-8}$ M. As can be seen, by utilizing the pulse mode, better results were not obtained, due probably to the irreversibility of the reduction process of the piroxicam.

Adsorption-Controlled Process. Because Viré et al. (1) found an adsorption phenomenon in the case of piroxicam on a mercury electrode, several experiments were carried out and a rapid and sensitive electroanalytical method is proposed. For this, the following solution was prepared: 15 μl of 10^{-4} M piroxicam in acetonitrile, 5 ml of 0.5M KNO_3, 5 ml of acetonitrile, 5 ml of 1 M acetic acid/acetate buffer and deionized H_2O to make 25 ml.

Scan Rate Influence. With a 2×10^{-7} M piroxicam solution in acetonitrile/H_2O, (20%) 0.05 M H_2SO_4 (pH 1.0) or HAcO/AcO^- (pH 4.7), and 1 M KNO_3, the influence of the scan rate on peak height was also studied.

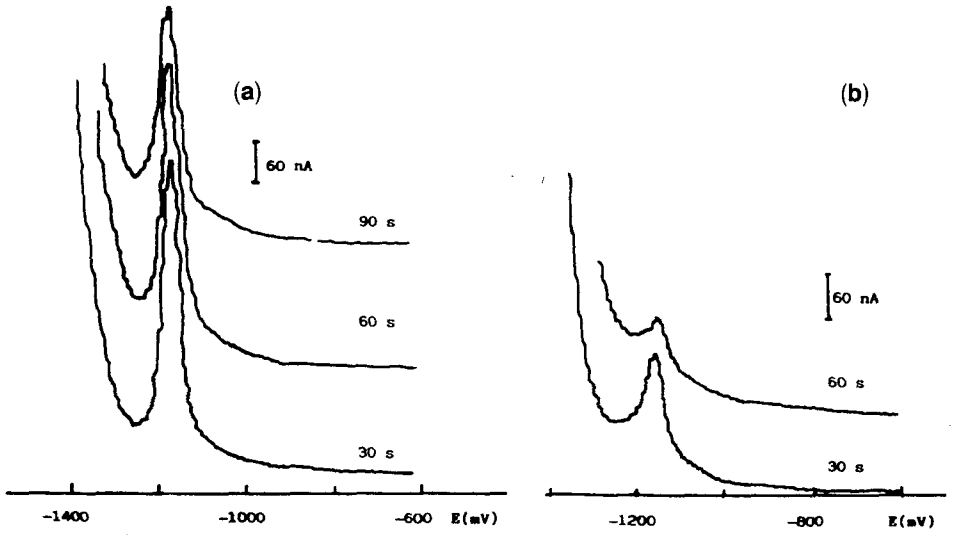

FIGURE 7. Influence of ad-accumulation time and ad-accumulation potential on voltammetric peak heights of 6 × 10^{-7} M piroxicam in acetonitrile/H_2O(20%), pH 4.7: (**a**) ad-accumulation potential, 0 V and (**b**) ad-accumulation potential, −0.6 V.

Two straight lines were obtained whose equations were the following:

$$\text{(pH 1.0)} \quad i_p = 0.4 + 0.77v; \quad r = 0.9993$$

$$\text{(pH 4.7)} \quad i_p = 5.74 + 1.17v; \quad r = 0.9990$$

where i_p denotes the peak height in nanoamps and v the scan rates in millivolts per second. According to Laviron (20), by measuring the slopes of these straight lines the r^* could also be calculated very high values (at pH 1.0, 0.77 mmol/cm^2 and at pH 4.7, 1.17 mmol/cm^2) were obtained when compared to the qualitative observations carried out by other authors (1).

Ad-Accumulation Time Influence. In this solution, the piroxicam concentration was 6 × 10^{-8} M. The piroxicam on the HMDE at 0.0 V (at open circuit) was preconcentrated, and the potential scanned between −1000 and −1500 mV at 100 mV/s (Figure 6). An accumulation process was observed, because the longer the ad-accumulation time, the higher was the peak. Figure 6 shows that the background presents a wave-shaped signal at about −1300 mV and that the piroxicam presents a peak at about −1350 mV. Taking into account the background current, the total heights were measured and by subtracting this background current and plotting i_p versus t (ad-accumulation time), a straight line was obtained, whose equation was the following:

$$i_p = 1.12 + 0.133t$$

In order to improve the results obtained in the case of the diffusion process mentioned above, a study of the piroxicam determination was carried out by using adsorptive stripping voltammetry (differential pulse mode).

For this, the following acetonitrile/H_2O(20%) solution was prepared: 6 × 10^{-7} M piroxicam, 0.1 M KNO_3, 0.1 M HAcO/AcO^- (pH 4.57). With this solution simultaneously, the ad-accumulation potential (at open circuit, 0.0 V, as well as at −0.6 V) and ad-accumulation time influence were studied, utilizing in all of the cases a ΔE = −50 mV. The results obtained are plotted in Figure 7, in which one can see that the best results were the ones obtained at open circuit potential (0.0 V) and that times longer than 30 seconds of preconcentration were not very appropriate.

In this sense, utilizing an ad-accumulation time of 30 seconds and 30 seconds of rest period and adding volumes of 15 μl of 1.05 × 10^{-4} mol/L piroxicam, the following equation was obtained:

$$i_p = 1.50 + 6.62 \times 10^9 C$$

where i_p denotes the peak heights in nanoamps and C the piroxicam concentration in mols per liter and whose detection limit was 2.44 × 10^{-11} M and correlation coefficient was 0.9991, showing that the adsorptive stripping voltammetric method established for piroxicam was highly sensitive, due to the strong adsorption processes characterized by Viré et al. (1) and studied by us.

CONCLUSIONS

This article is one of a series about the electroanalytical behavior of several organic molecules, such as analgesics [24,25] antiinflammatories [26,27], and azo-dyes [28,29], carried out in our department. In the case of piroxicam, this article confirmed the observations of Viré et al. [1] and "has added" to the knowledge about this drug by using polarographic and voltammetric techniques to elucidate the piroxicam electroreduction mechanism based on experimental observations. In addition, a very sensitive analytical method of this drug (detection limit of 2.44 × 10^{-11} M) is proposed by using adsorptive stripping voltammetric techniques whose analytical applications, alone, as well as in combination with modified electrodes, are of great interest in the area of organic molecules determination, as can be seen in the literature of the last 10 years.

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