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ELECTROCHEMICAL STUDIES USING ACTIVATED GLASSY CARBON. II. PIROXICAM

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The piroxicam was investigated by using CV and DPV techniques at an anodically activated glassy carbon electrode (GCE) in water-methanol solution. Different times for application of the anodic activation electrode potential were used and different times for application of different accumulation electrode potentials were used in each case. The anodically activated GCE leads to a better electrochemical behaviour (much better defined and more stable peak currents) regarding both peak current heights and peak potential values in comparison with the behaviour of piroxicam at the inactivated bare GCE.

INTRODUCTION

Oxicams as meloxicam, piroxicam and tenoxicam are enolic acid derivatives belonging to the non-steriodal anti-inflammatory drugs. These drugs are a group of diverse chemical composition and different therapeutic activities but three common features: identical basic pharmaceutical properties, similar basic mechanism of action, and similar adverse effects.[1,2] They exhibit acidic properties due to their enolic structure with pK_a in the range of medium acidic strength (3-5). The enolic structure is preferred due to the greater extent of conjugation.[3] In the gastric juice they act in their protonated form but in the plasma they are highly ionized due to their amphiphilic properties.

Oxicams derive from benzene(thieno)thiazine heterocyclic system, where the group of N-heterocyclic carboxamide include the triazine sulphur, and in position 4 there is an enolic group. Condensation of benzene ring or thiophene ring with the heterocyclic system as well as substitution of the amide group in position 3 imparts acidic properties to the enolic group. For their redox properties, amide and enol groups seem to be responsible.[4] The oxicams were studied by using especially polarographic methods in their different variants[5-8] or by adsorptive stripping CV at GCE.[4] Piroxicam (see Fig. 1) is the prototype of this class of drugs well-known as oxicams [9,10].

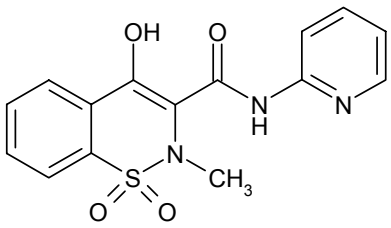

Fig. 1 – Piroxicam structure.

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The electrochemical response of piroxicam at solid electrodes is not too defined. To improve the response, as concern both the anodic peak current height and the anodic peak potential position, it is necessary to anodically activate the GCE. Using the procedure described, 11 one can improve the electrochemical response of many biological compounds, by increasing both the activity and reproducibility of carbon electrodes.12-14 In addition, at the pretreated GCE some biological compounds can be adsorbed and, as a consequence, accumulated at the electrode surface prior to CV and/or DPV experiments. Anodic activation of the surface of GCE at high anodic electrode potential results in an oxidized film containing functional group, especially of carbon-oxygen type (maybe of quinone type7). As a result the number of active sites at the GCE surface increases and the electron transfer reaction can be improved. [8,15-17]

EXPERIMENTAL

Apparatus: Electrochemical experiments were carried out using the potentiostat-galvanostat system AutoLab PGStat 12, controlled by General Purpose Electrochemical System (GPES) electrochemical interface for Windows (version 4.9.007). Three electrodes in one-compartment cell (10 ml) were used in all experiments. Glassy carbon electrode served as a working substrate electrode. All potentials were measured and given referred to SCE electrode used as reference electrode. The counter electrode was a rod glassy carbon electrode.

Measurements: All measurements were carried out at room temperature. All solutions were deaerated by dry argon stream for 5 min before every experiment and an argon atmosphere was maintained above the solution during the experiment

Chemicals: All chemicals were reagent grade, methanol (Chimopar), the components of the phosphate buffer solution (Carlo Erba) and the oxicams as piroxicam (high purity kindly offered by *LaborMed Pharma S.A.*- from manufacturer) were used without further purification; the aqueous solutions have been prepared using doubly-distilled water. Before modification, the glassy carbon surface was polished with alumina slurry on a polishing pad, washed with distilled water and sonicated for 3 minutes in doubly distilled water.

RESULTS AND DISCUSSION

Fig. 2 shows the typical cyclic voltammograms for 10^{-4} M piroxicam at 50 mV/s scan rate at inactivated (bare) GCE, at activated GCE at 1.8 V (activation electrode potential) for 60 s and respectively for 120 s activation time, but at the same preconcentration/accumulation electrode potential and the same preconcentration/ accumulation time. Piroxicam was dissolved into a solution obtained by mixing methanol (1 v) and PBS of pH 7 (4 v), of course the components of PBS acting also as indifferent electrolyte.

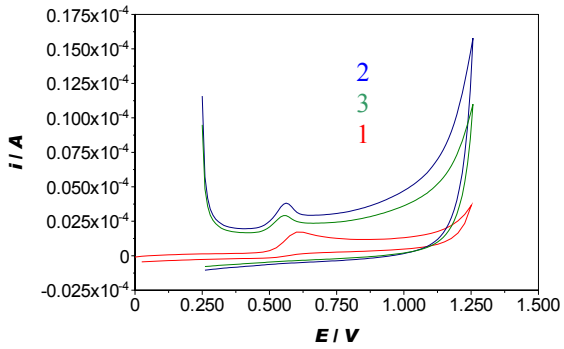

Fig. 2 – Comparison between three cyclic voltammograms (scan rate of 50 mV/s, potential range from 0.25 to 1.25 V) illustrating the different electrochemical responses of an inactivated GCE (line 1) and of an activated GCE at different values of activation time (60 s – line 2 and 120 s – line 3), for an activation electrode potential of 1.8 V and an accumulation/preconcentration electrode potential of -0.7 V (with an accumulation/preconcentration time of 40 s).

The effect of anodic activation of a bare GCE, at 1.8 V (vs. SCE) either for 60 s (Fig. 2, curve 2) or for 120 s (Fig. 2, curve 3) and of a preconcentration/accumulation electrode potential of – 0.7 V applied for 40 s, can be seen in Fig. 2, for 50 mV/s scan rate (Fig. 2, curve 1, the bare GCE response). For bare (inactivated) GCE the anodic peak potential is close to 0.635 V while for both anodic activation time used a negative shift is noticed, for 60 s activation time the anodic peak potential shifts to 0.542 V (the peak potential shift being -0.093 V) but for 120 s activation time only to 0.572 V (the peak potential shift being also negative, -0.063 V) for an accumulation electrode potential of -0.7 V applied for a duration of 40 s, as can be seen in Fig. 2. For the example given in Fig. 2, the peak current ratio is 2.19 for an activation time of 60 s and 2.79 for an activation

time of 120 s, an enhancement due to the increase of the activity of the anodically activated GCE at 1.8 V versus SCE. The electroactive species gives rise to a much better defined cyclic voltammogram having both peak currents increased and peak potentials anticipated in comparison with the response recorded for an inactivated GCE. It can be noticed that simultaneously the hysteresis of the cyclic voltammogram at GCE modified by anodic activation increases as it is usually expected for chemically modified electrodes. The activation of GCE for 60 s seems to lead to a better electrochemical response (as regarding definition of the peak, as regarding the peak potential position, but not as regarding the peak current height) in comparison with the activation at 120 s duration.

Fig. 3 shows the cyclic voltammograms for 10^{-4} M piroxicam at 50 mV/s scan rate at activated GCE at 1.8 V (activation electrode potential) for 60 s activation time, but at three different accumulation electrode potentials applied for the same preconcentration/accumulation time. The explored range of electrode potentials (versus SCE) was from 0.25 V to 1.25 V at a bare (inactivated) GCE and, also, at anodically activated GCE. In this explored potential range there is only one anodic peak which, at this pH, appears at 0.635 V for bare (inactivated) GCE. On the reverse scan there is no complementary cathodic peak.

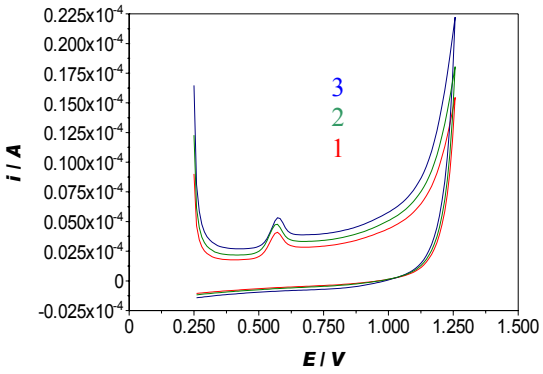

Fig. 3 – Comparison between three cyclic voltammograms (scan rate of 50 mV/s, potential range from 0.25 to 1.25 V) illustrating the influence of value of the accumulation/preconcentration electrode potential (-0.5 V (line 1), -0.6 V (line 2), -0.7 V (line 3)) maintained for 60 s. The activation electrode potential is 1.8 V applied for 60 s.

The anodic peak potential, considered here as a mean value for all six accumulation time used, varies with decreasing accumulation potential from 0.532 V (for -0.5 V) to 0.539 V (for -0.6 V) to 0.538 V (for -0.7 V), in the case of an anodic activation applied for 60 s. For 120 s activation time, the anodic peak potential had a different evolution, from 0.572 V (for -0.5 V) to 0.562 V (for -0.6 V) to 0.577 V (for -0.7 V). But the peak current increases constantly with decreasing accumulation potential at each accumulation time used due to the increasing cathodic overvoltage applied with decreasing accumulation electrode potential applied.

The behaviour of 10^{-4} M piroxicam at an inactivated GCE was studied in the scan rate range from 25 to 500 mV/s (25, 50, 75, 100, 200, 300, 400 and 500 mV/s) and all the cyclic voltammograms obtained have the typical shape shown in Fig. 2, curve 1. The usual interpretations (both plots I_p versus $v^{1/2}$ and $\ln I_p$ versus $\ln v$) are consistent with a linear behaviour, both of them having a correlation coefficient of 0.993, for the second one a slope of 0.51 was obtained, showing, together with the constancy of the anodic peak potential (there is only a slight variation around 0.635 V), a reversible behaviour (diffusionally controlled electrode reaction). Of course, the absence of the complementary cathodic peak, on the reverse scan, in all the range of scan rates studied, is due to the existence of very fast chemical step(s) following the charge transfer step(s), chemical step(s) giving rise to electroinactive chemical compound(s). As was shown,[18,19] the appearance of the anodic peak supposes a two-electron charge transfer step [18,19] of practically the same standard electrode potential. [19] So that it is possible as two very fast following electrode reactions to be involved in the occurrence of the anodic peak and responsible for the lack of the cathodic peak. At this almost neutral pH the hydroxyl group exists as such but the nitrogen from pyridine moiety is protonated. So the

mechanism involves a first loss of an electron from the hydroxyl group lone pair followed by a very fast deprotonation at the radical cation hydroxyl group leading to a radical. This O radical is the subject of a second loss of an electron (occurring either at the same or at a less positive standard electrode potential, *i.e.*, easier), then followed by an electronic rearrangement leading to a β-diketone with the carbocationic character at the in between carbon atom. This carbocation intermediate is subjected to a nucleophylic attack of a water molecule (or methanol) with the formation of 2-hydroxy-1,3-diketonic (or 2-methoxy-1,3-diketonic) structure which is electroinactive. This last product decomposes, in several unknown steps, including a hydrolysis and a homogeneous oxidation addition, to the ortho-amino-pyridinium cation and another product. [19] The same mechanism seems to be followed at the anodically activated GCE irrespective of both the activation time (60 s or 120 s) used and of the preconcentration/accumulation electrode potential (-0.5 V, -0.6 V and -0.7 V) and applied time (from 0 s to 60 s with a step of 10 s) used due to the fact that the cyclic voltammograms have the same basic shape.

For an activation time of 60 s (see Fig. 4 A) and of 120 s (see Fig. 4 B), at activation potential of +1.8 V (vs. SCE), the preconcentration/ accumulation time has been changed in the range from 10 s to 60 s, with a step of 10 s, and the cyclic voltammograms were recorded at 50 mV/s and the differential pulse voltammograms were recorded at PA=25 mV and SP=5 mV. The anodic peak current is well-shaped in both cases, but even better defined for 120 s accumulation time than for 60 s accumulation time. The definition is better and better with increasing accumulation time. Again, there is no cathodic peak on the cathodic sweeping of the electrode potential.

Also, the DPV (see Fig. 5) studies lead to the same conclusions regarding the difference between the behaviour at the two different activation durations used and the better definition of the peak shape at the larger activation duration in comparison with the smaller one. The parameter used for DPV experiments were: pulse amplitude of 25 mV and step potential of 5 mV. The DPV studies were also performed for the anodic activation of the GCE at an electrode potential of 1.8 V (versus SCE) at both activation time (60 and 120 s) and for all three accumulation electrode potentials -0.5 V, -0.6 V and -0.7 V (versus SCE) at each accumulation time (from 10 s to 60 s, with a step of 10 s) used.

The accumulation time influence on the peak currents and peak potentials was also studied at another two accumulation electrode potentials, -0.6 V and, respectively, -0.7 V. Both peak currents increase and peak potentials shift in the negative direction occur at any accumulation time (from 10 to 60 s, with a step of 10 s) used for the accumulation of the compound and at any activation time (60 s and 120 s) used for anodic activation of the bare GCE. In each case, the peak currents increase with the increasing application time of the accumulation potential for an accumulation time in the range from 0 s to 60 s, showing an electrocatalytic action of the activated bare GCE. In all three cases considered above, the increases of the two anodic peak currents were either linear (i.e., with a constant slope) or tend to lower (i.e., the curve increases with a decreasing slope).

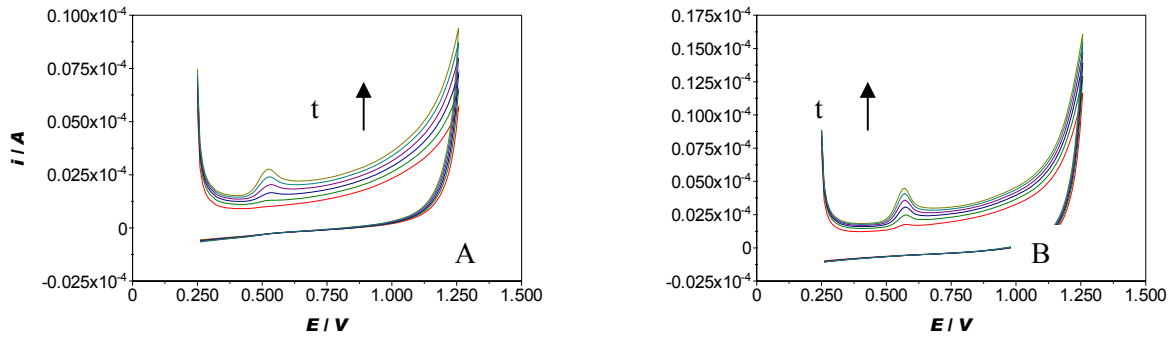

Fig. 4 – Cyclic voltammograms illustrating the dependence on the GCE accumulation/preconcentration time (10, 20, 30, 40, 50 and 60 s) at the preconcentration potential (-0.5 V), recorded at 50 mV/s scan rate, 1.8 V activation electrode potential for 60 s (A) and for 120 s (B), range of potential 0.25 – 1.25 V.

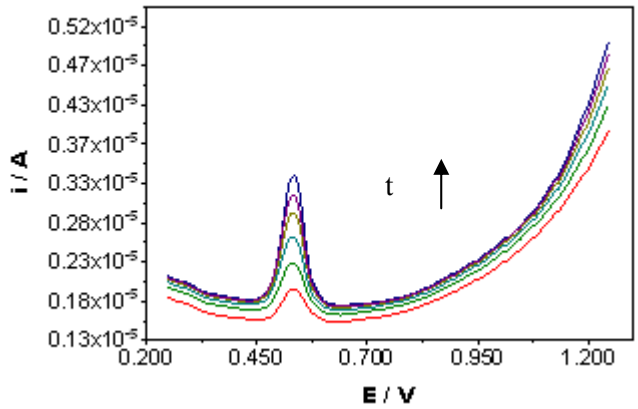

Fig. 5 – DPV-traces illustrating the dependence on the GCE accumulation/preconcentration time (10, 20, 30, 40, 50 and 60 s) at the preconcentration potential (-0.5 V), recorded at 50 mV/s scan rate, 1.8 V activation electrode potential for 120 s, range of potential 0.25 – 1.25 V, PA=25 mV and SP=5 mV.

The influence of different accumulation time (from 0 s to 60 s, with 10 s time step) on the peak currents and peak potentials is given in Table 1, for anodic activation at 1.8 V but for two different duration of the activation, 60 s, and, respectively, 120 s. In both cases, the accumulation electrode potential was -0.7 V. As can be noticed, the peak currents increase with increasing accumulation time and the peak potential is either almost constant or varies slightly anodically for both activation durations of GCE. In the linear case behaviour, one may affirm that the amount of the accumulated piroxicam in the interfacial region increase linearly with the increasing accumulation time, while for the curved case of decreasing slope it is possible as, in the time scale of the cyclic voltammetry experiments, especially at longer duration of accumulation, an equilibrium between the diffusional and the adsorbed concentrations of the electroactive compound to be installed. In the range of explored accumulation time, no decrease in the peak current occurs, so it seems probably that the saturation coverage of the electrodic interface is not yet obtained.

Table 1

Peak currents and peak potentials at different accumulation time but the same accumulation potential (-0.7 V) for two different anodic activation times (60 s, and, respectively, 120 s) but the same anodic activation electrode potential (1.8 V). Data are read from both CV and DPV studies

	Activation time 60 s CV DPV						Activation time 120 s CV DPV					
t (s)	10	20	30	40	50	60	10	20	30	40	50	60
E_{pa}	0.522	0.532	0.542	0.542	0.542	0.552	0.582	0.582	0.582	0.572	0.572	0.572
(V)	0.542	0.544	0.544	0.542	0.541	0.545	0.555	0.552	0.552	0.551	0.550	0.548
I_{pa}	2.30	2.79	3.39	3.75	4.12	4.52	2.68	3.52	4.17	4.76	5.29	5.74
(μA)	3.34	3.72	4.10	4.39	4.63	4.86	3.61	4.15	4.55	4.82	5.17	5.32

The accumulation time influence of the peak currents and peak potentials was also studied at another two accumulation electrode potentials, -0.5 V and, respectively, -0.6 V. Both peak currents increase and peak potentials shift in the negative direction occur at any accumulation time used for accumulation of the compound and at any activation time used for anodic activation of the bare GCE. In each case, the peak currents increase with the increasing application time of the accumulation potential for an accumulation time in the range from 0 s to 60 s, showing an electroactive action of the activated bare GCE. In all three cases considered above, the increase of the anodic peak current was either linear (i.e., with a constant slope) or tended to lower (i.e., the curve increases with a decreasing slope).

The results of the different accumulation electrode potentials used in cyclic voltammetry and differential pulse voltammetry studies are summarized in Table 2.

Table 2

Peak currents and peak potentials at different accumulation potentials (-0.5 V, -0.6 V and, respectively, -0.7 V) for 60 s accumulation time for two different anodic activation times (60 s, and, respectively, 120 s) but the same anodic activation electrode potential (1.8 V). Data are read from both CV and DPV studies

	Activation duration 60 s CV/DPV			Activation duration 120 s CV/DPV		
E (V)	-0.5 V	-0.6 V	-0.7 V	-0.5 V	-0.6 V	-0.7 V
E_{pa} (V)	0.522/0.528	0.532/0.531	0.542/0.540	0.572/0.548	0.572/0.548	0.572/0.548
I_{pa} (μA)	2.71/4.43	3.92/4.68	4.52/4.75	4.47/4.51	4.76/5.14	5.74/5.32

The results obtained by CV are consistent with those obtained by DPV experiments as concerning the evolution of both the peak potentials and peak currents.

CONCLUSIONS

In order to obtain a better electrochemical response to a cyclic voltammetry perturbation, it is customary to improve the electrochemical behaviour of a bare GCE. The choice for the enhancement of the electron transfer ability was to anodically activate the surface of the GCE with the obtaining of an oxidized film containing functional groups, especially of carbon-oxygen type, which confer an improved redox activity, and having a larger active area due to its increased porosity. Both the enhancement of peak currents and the cathodic shift of the anodic peak potentials were obtained at the anodically activated GCE versus inactivated bare GCE. Also a better peak definition resulted. Both the accumulation electrode potential (-0.5, -0.6 and -0.7) influence and different accumulation time (from 10 s to 60 s, with an increase of 10 s) influence, for the same accumulation electrode potential, were explored for an anodic activation made either for 60 s or for 120 s activation time. A more and more cathodic accumulation potential increases the anodic peak current for the same activation time but the evolution of the position of the anodic peak potential is different for each activation time used. As resulted from the comparison between the cyclic voltammograms, the activation time of 60 s leads to a higher anodic peak current but to a less anticipated anodic peak in comparison to the activation at 120 s. For each activation time, the peak currents increase with increasing cathodic accumulation electrode potential and also with increasing accumulation time of piroxicam.

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