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Electrochemical behaviour of droxicam: kinetic study in aqueous-organic media

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Abstract

A kinetic study of the hydrolytic decomposition of droxicam was carried out in order to establish the possible pharmacological action of the drug in the organism of the human being. The electrochemical technique used was scan cyclic voltammetry on a HMDE, giving a well-defined peak. Due to the insolubility of droxicam in water, the working media was a methanol–H_2O (4:96, V/V) mixture. The decomposition product of droxicam is piroxicam, which is also reduced on a mercury electrode giving a well-defined cyclic voltammetric peak. It is proposed that the decomposition of droxicam is controlled by a kinetic equation: $v = (1.93 \cdot 10^{-3} + 78.9\ [D])\ [D] = k_D[D]$.

Keywords: Droxicam; Kinetic study; Organic-aqueous media

1. Introduction

Droxicam and piroxicam are two active principles of the oxicam's family constituted for *N*-carboxamilic heterocycles derived from benzothiazine-1,2-dioxide. These substances have been employed in the manufacture of non-steroidian anti-inflammatory drugs, as well as analgesic drugs, mainly due to the prostaglandin biosynthesis 'in vivo and in vitro', as well as in the inhibition of the phagocyte activity and the leukocyte migration [1].

These molecules are scarcely soluble in water. Nevertheless, they can be dissolved in organic solvents, in aqueous-organic and micellar media [2].

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Reduction of piroxicam on a mercury electrode [1] and its oxidation on a carbon electrode [2,4,5], have been widely studied by different authors and several methods to its electroanalytical determination have been proposed [2,3,6,7].

Regarding droxicam, 5-methyl-3-(2-pyridil)-2H,5H-1,3-oxacino(5,6-C)-(1,2)benzo-thiazine-4,4(3H)-diona-6,6-dioxyde, no reference to its electrochemical behaviour has been reported, although several papers concerning its anti-inflammatory properties and gastrointestinal tolerance, as well as its behaviour in the organism as a pre-drug of piroxicam, due to its best assimilation, can be found in the literature [8–11].

The pharmacokinetics and metabolism of droxicam have been studied by Farré et al. [9] and Esteve et al. [11]. In the latter paper, comparative studies of the properties and the anti-inflammatory activity of droxicam and piroxicam were carried out, showing that these drugs are much more active than phenylbuthazone.

With respect to the effects of droxicam on the prostaglandin synthesis, Esteve et al. [11] affirms that they are similar to that of the piroxicam. In this regard, Palacios et al. [12] carried out a study by electronic microscopy of the harmful effects of piroxicam and droxicam on rat tissue.

Regarding human samples, Martínez et al. [13] carried out several studies on the tolerance of droxicam by voluntary patients.

On the other hand, Frigola [14] carried out a complete structural study by RMN, SM and RX on droxicam.

Finally, as droxicam is utilized for chronic ill treatments as a pre-drug of piroxicam, in order to avoid the adverse effects of this drug, we think that it is interesting to carry out a kinetic study on the transformation of droxicam in piroxicam. On the other hand, the utilization of electrochemical techniques for monitoring this kinetic is a novelty, because, for example, the use of HPLC was impossible to employ. Moreover, the proposed method is simple, fast, inexpensive and without additional sample preparation.

2. Experimental

2.1. Apparatus

Electrochemical measurements were carried out using a PSTAT10-AUTOLAB Potentiostat Eco-Chemie (The Netherlands) with GPES software.

The utilized electrodes were: a HMDE of Metrohm 6.1230.010 as working electrode, an Ag/AgCl, KCl sat. Ingold 9823 as reference electrode and a Pt wire as auxiliary electrode. The electrochemical cells were Metrohm 6.1415.210 and Metrohm EA 876-20. For the temperature control, a thermostat Tamson TC 3 with water circulation was used.

2.2. Reagents

Droxicam and piroxicam (pure active principle) were furnished by Pfizer Laboratories. The working solutions were prepared in pure methanol (Merck) at a 1.25 mM droxicam concentration.

All reagents were of analytical grade (Merck) and were used without further purification.

The used water was deionized up to nanopure grade with the Barnstead system.

2.3. Procedures

Working solutions were prepared by mixing 1 ml of 1.25 mM droxicam solution in pure methanol with buffer solution in a volumetric flask to make 25 ml. The final methanol–H_2O ratio was (4:96, V/V), except in the case in which the variable parameter was the methanol–H_2O ratio.

These solutions were studied by cyclic voltammetry between -0.6 and -1.3 V at a constant temperature of 25 °C.

3. Results and discussion

To carry out this study, the first step was to verify that droxicam, due to its similar behaviour to that of piroxicam, gave an electrochemical

signal in reduction. Thus, by cyclic scan, at 0.2 V s^{-1} on a HMDE (surface 1.14 mm^2), a 50 μM droxicam solution in methanol–H_2O (4:96, V/V) medium, at pH 4.0 (0.1 M Briton–Robinson buffer solution), gave the voltammogram plotted in Fig. 1(a).

This peak must correspond to the electrochemical reduction given in Fig. 1(b), because, as we can verify, its shape is similar to that of the piroxicam given in the literature [1,4,5].

As we know, to propose an electrochemical reaction of a non-studied compound, it is necessary to calculate the number of electrons transferred by the molecule. This parameter is normally calculated by coulometric methods at constant potential. As droxicam is not soluble in water, it must be dissolved in aqueous/organic media or in pure organic solvents. In aqueous/organic media, this drug decomposes with time and in pure solvent it is stable, but the utility of droxicam as a drug is in biological media, where there is water, other molecules and tensioactive substances to solubilize more or less lipidic molecules. Thus, we are obliged to carry out our study in a medium where droxicam decomposes gradually. Therefore, it is impossible to determine the number of transferred electrons by a coulometric method and we must suppose that this

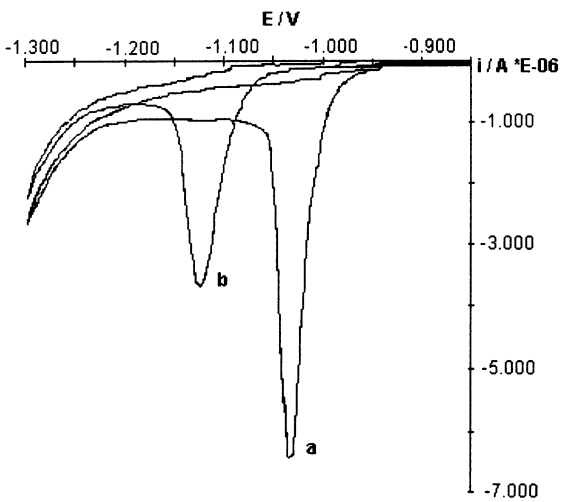


Fig. 1. Cyclic voltammograms (a) 50 μM droxicam solution; (b) 50 μM piroxicam solution in methanol–H_2O (4:96 V/V); pH = 4 Briton Robinson buffer and a scan rate of 0.2 V s^{-1}.

number is 2, as in the cases of other oxicams of similar structure, such as piroxicam or tenoxicam.

3.1. Electroanalytical study versus time

In the following experiment, the droxicam I_p versus time, at constant temperature, was measured by cyclic voltammetry. Therefore, several droxicam solutions at different concentrations in methanol–H_2O (4: 96, V/V) media at pH 4.0 (Britton–Robinson 0.1 M solution), were prepared.

For each solution, cyclic voltammograms were recorded for 6 h, the peak heights were measured every 30 s for the first 10 min and every 15 min thereafter.

Between each two measurements a nitrogen gas current was bubbled through the solutions to eliminate oxygen. The cell was thermostatted at 25 °C and protected against the light throughout the process.

Initially, only the droxicam reduction peak, at −1.033 V (Ag/AgCl, KCl), was observed. With time, this peak decreased and a new peak appeared at −1.124 V (Ag/AgCl, KCl), that increased, while the first one gradually disappeared. The second peak is due to piroxicam produced by decomposition of droxicam. To verify this, several additions of a piroxican solution were made and progressive increases of the second peak were obtained. This confirmed that droxicam is transformed to piroxicam in solution [9,10].

In Fig. 2, three voltammograms are shown, the first voltammogram (a) was obtained at the beginning of the experiments, another voltammogram (b) after 120 min, where it can be observed that the peak corresponding to the droxicam decreases whereas, at the same time, one peak due to the piroxicam generated from droxicam appears and finally, the voltammogram (c) corresponding to an added piroxicam solution, under the same experimental conditions.

The evolution of peak intensity of each species (droxicam and piroxicam) versus time is plotted in Fig. 3. In this experiment, the initial droxicam concentration was 40 μM, and the other parameters are the same as in Fig. 2. Therefore, in Fig. 3, it can be easily verified that piroxicam is formed at the expense of droxicam.

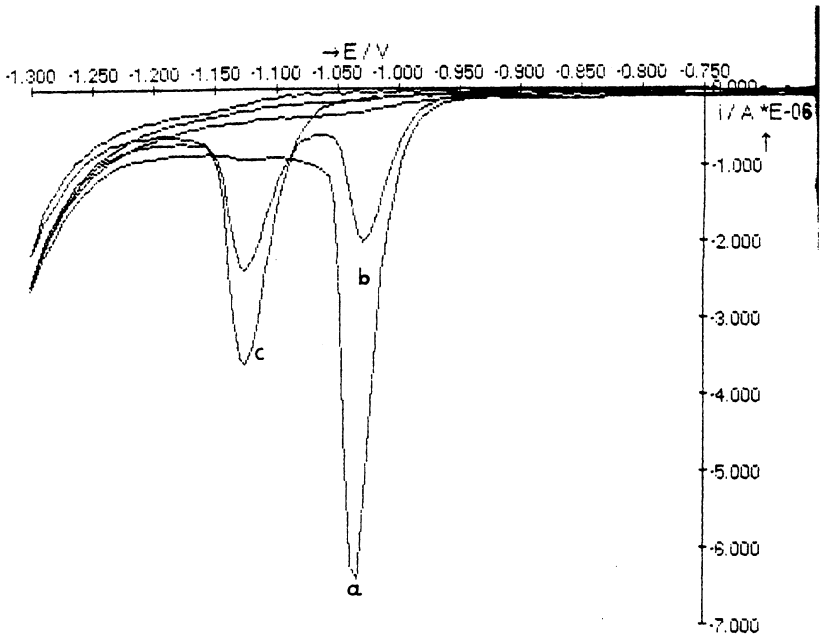


Fig. 2. Cyclic voltammograms at different times of: (a) 40 μM droxicam solution, at the beginning; (b) after 120 min; and (c) 40 μM piroxicam solution added. Experimental conditions are the same as in Fig. 2.

3.2. Influence of initial droxicam concentration, pH and methanol–H_2O (V/V) ratio

This experiment was carried out over 2 h and consisted of several measurements of droxicam peaks, corresponding to solutions where initial droxicam concentration, the pH and methanol–H_2O (V/V) ratio, was varied, in order to determine the influence of these parameters in the droxicam decomposition to propose a possible way for its transformation.

With the data obtained above for short experimental times, a reaction order of 1.23 was calculated for the droxicam decomposition. Because this order is not exact, it was approximated to the unity [15–17]. Therefore, the evolution of droxicam decomposition could follow an equation of first order that is of easy integration due to a decomposition mechanism.

By plotting $\ln\{[D]_0/[D]\}$ versus t for every experiments, where $[D]_0$ and $[D]$ are the droxicam concentration at zero time and t time, respectively, we must obtain a straight line whose slope furnishes the k_D value for every case [15–17].

The $[D]$ values that represent the droxicam concentration versus time were calculated from a calibration graph, previously obtained, by plotting the peak intensity versus droxicam concentration because the followed parameter was the droxicam reduction peak intensity.

The calibration equation of the straight line is:

$$I_p(\mu A) = 0.048 + 0.200 \cdot 10^6 C(M) \quad r^2 = 0.992$$

with a linear concentration range between 8.10^{-7} and 5.10^{-5} M. The detection limit calculated on the basis of IUPAC definition was $7.91\ 10^{-7}$ M = 0.283 ppm.

Validation of this procedure was carried out by the cyclic voltammetry technique to analyte solutions containing known analyte amounts, by means of recovery experiments, finding an average recovery percentages of $98.33 \pm 2.53\%$ for the same concentration range indicated above.

3.2.1. Influence of droxicam initial concentration

Experiments, where the variation of I_p versus droxicam solutions with different initial concentrations, were carried out. In these solutions, the

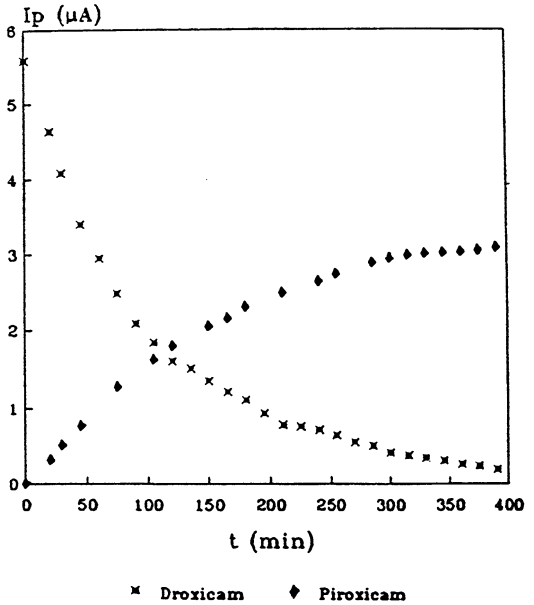


Fig. 3. Evolution of droxicam and piroxican intensity peaks versus the time, 40 μM initial droxicam concentration. Experimental conditions are the same as in Fig. 2.

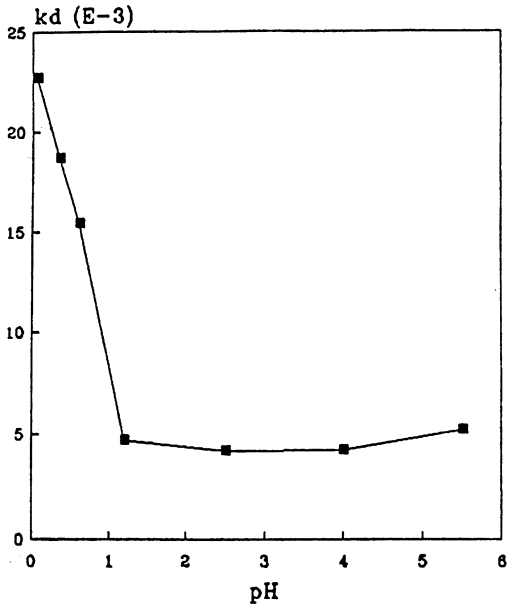


Fig. 4. Variation of k_D value versus pH. Droxicam: 30 μM, in methanol–H_2O (4:96, V/V).

pH value was 4 and the ratio methanol–H_2O was (4:96, V/V). The used potential scan rate was 0.2 V s^{-1} and the constant k_D values were calculated as indicated above. These k_D values are summarized in Table 1.

By plotting the k_D values versus $[D]$, a straight line, with the following equation:

$$k_D = 1.93 \cdot 10^{-3} + 7.90[D](M)$$

and a correlation coefficient with a value of 0.995 was obtained.

This equation gives a term of order 1 and another one of order 0 versus droxicam initial concentration.

Table 1
Variation of k_D values with the initial droxicam concentration

$[D]_0$ (μM)	k_D (10^{-3})
5	2.43
10	2.70
20	3.52
30	4.24
40	4.95
50	6.06

3.2.2. pH influence

In this experiment, a study of pH influence on a 30 μM droxicam solution in methanol/H_2O (4/96) (V/V) ratio was carried out. pH values were between 0.06 and 5.50 and obtained by using 1 and 0.1 M HNO_3 solutions as well as 0.1 M Britton–Robinson solutions. Basic pH values were discarded because piroxicam gives very poor signals.

The obtained values for k_D are plotted in Fig. 4.

As can be appreciated, the acidity of media is determinant in the droxicam chemical reaction. Thus, the lower the pH, the higher the k_D calculated values, the more rapid a reaction obtained. To pH values higher than 1.2, the k_D value varies very little, indicating that there is a specific acid catalysis.

3.2.3. Influence of methanol–H_2O (V/V) ratio

For this study, pH (4.0) and an initial droxicam concentration of 30 μM were maintained con-

Table 2
Variation of k_D values with methanol–H_2O (V/V) ratio

% Methanol	k_D (10^{-3})
4	4.24
8	3.49
12	3.06
16	2.32
20	2.07

stant. The methanol–H_2O (V/V) ratio was varied between 4 and 20%. The k_D calculated values for every case are summarized in Table 2.

As can be appreciated in Table 2, the higher the methanol amount, the lower the k_D values, indicating that the influence of the $\%H_2O$ is important. Therefore, the higher the $\%H_2O$, the higher the piroxicam transformation.

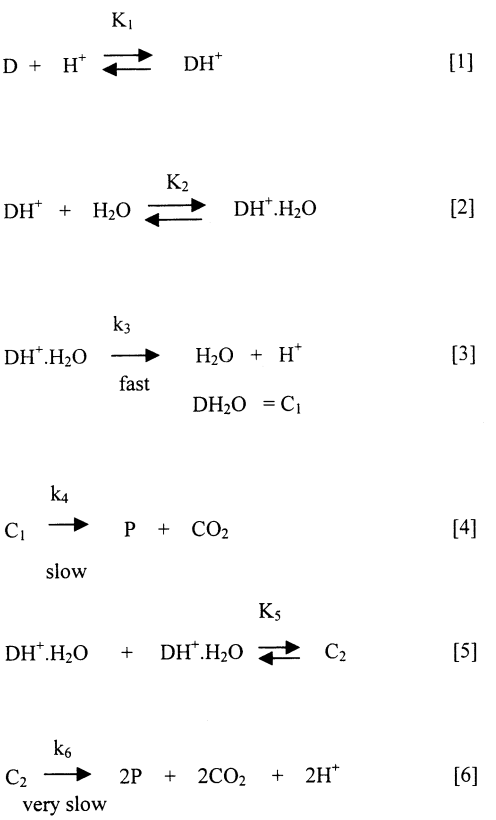
3.3. Proposition of a reaction kinetics

According to the obtained experimental results on the influence of droxicam initial concentration, the existence of a possible acidic specific catalysis, as well as the influence of the $\%H_2O$ of the medium, a possible way for the droxicam chemical decomposition is proposed, as can be seen in Fig. 5(a,b).

In Fig. 5 it can be seen that, starting with a droxicam acidic hydrolysis, piroxicam is produced following two possible ways through two different intermediate reactions.

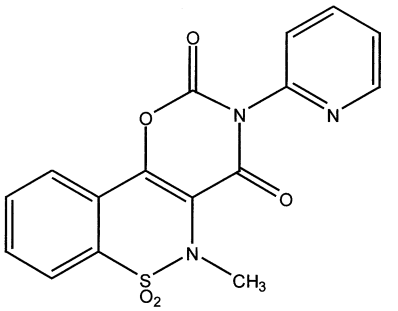
As a consequence of this mechanism, the reaction rate described as the piroxicam formation versus time can be written as follows

$$v = \frac{d[P]}{dt} = k_4[C_1] + k_6[C_2] \quad (1)$$

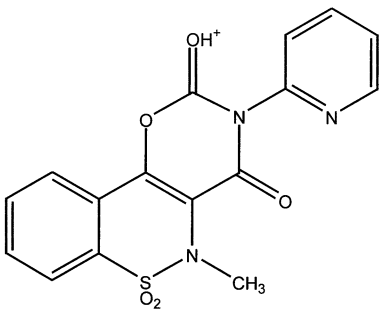


(a)

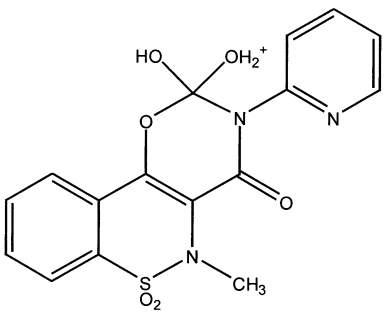
Fig. 5. (a) A possible way for the droxicam chemical decomposition. (b) Molecular formulae of all obtained products.



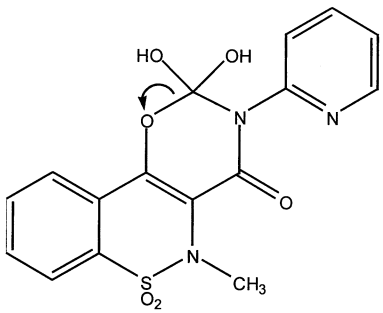
D



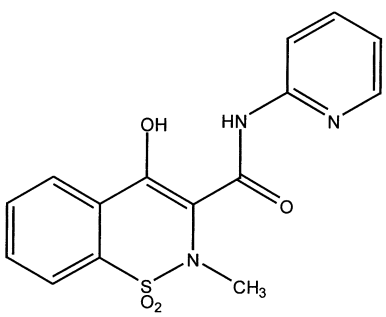
DH^+



$DH^+.H_2O$



$DH_2O = C_1$



P

(b)

Fig. 5. (*Continued*)

The reaction intermediate concentration calculus, C_1 and C_2, in order to justify the proposed equation ($k_D = 1.93 \cdot 10^{-3} + 78.9$ [D]), are described as follow.

3.3.1. Calculus of [C_1]

We must admit that species C_1 is an intermediate at the steady-state conditions. This is justified by its predictable low concentration, its unsuit-

ability and, therefore, a species of short life. By applying the steady-state approximation [15–17], we obtain:

$$\frac{d[C_1]}{dt} = k_3[D\mathrm{H}^+\cdot\mathrm{H_2O}] - k_4[C_1] = 0 \quad (2)$$

$$[C_1] = \frac{k_3}{k_4}[D\mathrm{H}^+\cdot\mathrm{H_2O}] = \frac{k_3}{k_4}K_2[D\mathrm{H}^+][\mathrm{H_2O}]$$

$$= \frac{k_3}{k_4}K_2K_1[D][\mathrm{H}^+][\mathrm{H_2O}] \quad (3)$$

To obtain $[C_1]$ we have used the equilibrium constants of Eq. (1) and Eq. (2). On the other hand, taking into account that medium is always buffered, $[\mathrm{H}^+]$ is constant.

3.3.2. Calculus of $[C_2]$

The step of Eq. (5) is, kinetically speaking, an Arrhenius type pre-equilibrium [15–17]. Consequently, the piroxicam generation by this way responds to the following equation:

$$k_6[C_2] = k_6K_5[D\mathrm{H}^+\cdot\mathrm{H_2O}]^2 = k_6K_5K_2^2[D\mathrm{H}^+]^2[\mathrm{H_2O}]^2$$

$$= k_6K_5K_2^2K_1^2[D]^2[\mathrm{H}^+]^2[\mathrm{H_2O}]^2 \quad (4)$$

and taking into account the piroxicam production rate, according to Eq. (6), the theoretical equation of the reaction rate is finally given by the following expression:

$$\frac{d[P]}{dt} = k_4[C_1] + k_6[C_2]$$

$$= k_3K_1K_2[D][\mathrm{H}^+][\mathrm{H_2O}] + k_6K_1^2K_2^2K_5[D]^2$$

$$\times [\mathrm{H}^+]^2[\mathrm{H_2O}]^2 = (a + b[D])[D] \quad (5)$$

where $a = 1.93\cdot10^{-3}$, $b = 78.9$ and $[D]$ in M.

4. Conclusions

It has been shown in this paper that droxicam decomposes with time in the presence of water giving piroxicam, which is another drug of the same family.

Taking into account that the decomposition product of droxicam is piroxicam, it is possible to carry out a study in order to establish a mechanism for this transformation.

As a result of our experiments, we have found that the decomposition reaction is an acidic hydrolysis, controlled by a velocity whose equation is the following:

$$v = \frac{d[P]}{dt} = (1.93\cdot10^{-3} + 78.9[D])[D] = k_{\mathrm{D}}[D]. \quad (6)$$

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References

[1] J.C. Viré, J.M. Kauffmann, J. Braun, G.J. Patriarche, Analysis 13 (38) (1985) 134–140.
[2] J.A. Acuña, Tesis de Licenciatura, Universidad de Valladolid, Noviembre, 1991.
[3] N. Abo El-Maali, J.C. Viré, G.J. Patriarche, M.A. Ghandour, G.D. Christian, Anal. Sci. 6 (1990) 245–250.
[4] J.M. Kauffmann, J.C. Viré, J. Gelbke, G.J. Patriarche, Anal. Lett. 17 (1984) 2319–2331.
[5] J.M. Kauffmann, A. Laudet, G.J. Patriarche, G.D. Christian, Talanta 29 (1982) 1077–1082.
[6] M. González, M.D. Vázquez, M.L. Tascón, P. Sánchez-Batanero, Electroanalysis 6 (1994) 497–504.
[7] A.R. Paniagua, M.D. Vázquez, M.L. Tascón, P. Sánchez-Batanero, Electroanalysis 6 (1994) 265–268.
[8] J. Esteve, Patent No. 4-563-452/1986.
[9] A.J. Farré, M. Colombo, M. Fort, B. Gutiérrez, L. Rodríguez, R. Roser, Meth. Find. Exp. Clin. Pharmacol. 8 (7) (1986) 407–422.
[10] Merck Index, eleventh ed., Merck and Co. Inc., Rahway, NJ, 1989.
[11] J. Esteve, L. Martínez, R. Roser, R. Sagarra, Meth. Find. Exp. Clin. Pharmacol. 9 (2) (1987) 121–126.
[12] G. Palacios, M. Colombo, R. Roser, J. Esteve, Meth. Find. Exp. Clin. Pharmacol. 9 (6) (1987) 353–370.
[13] L. Martínez, J. Sánchez, R. Roser, J. García-Barbal, A. Bartlett, J. Estruch, R. Sagarra, S. Puig, A. Costa, Meth. Find. Exp. Clin. Pharmacol. 10 (11) (1988) 729–737.
[14] J. Frigola, J. Chem. Soc. Perkin Trans. 2 (2) (1988) 241–245.
[15] G.M. Barrow, Química-Física, vol. II, Reverté, Spain, 1968.
[16] M. Díaz-Peña, A. Roig-Muntaner, Química-Física, vol. II, Alhambra, Spain, 1975.
[17] S.W. Benson, The Foundation of Chemical Kinetics, McGraw-Hill, USA, 1960.