A Selective Voltammetric Method to Follow the Hydrolytic Degradation of Nitrendipine and Nisoldipine

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Abstract

The development and application of a differential pulse voltammetric procedure for the hydrolytic degradation kinetic study of two well-known 1,4-dihydropyridine type drugs, nitrendipine and nisoldipine are presented. The DPV procedure exhibited an adequate selectivity, repeatability and reproducibility with CV lower than 2%. The recoveries were higher than 98% with CV of 1.63% and 1.87% for nitrendipine and nisoldipine, respectively. Hydrolysis of each drug was carried out in ethanol/Britton-Robinson buffer (30/70) at different pHs and at controlled temperature of 40, 60 and 80 °C. A significant degradation was observed at alkaline pH (> pH 8) following a first order kinetic for both drugs. At pH 12 the decay constant values of $4.85 \times 10^{-2} \text{h}^{-1}$ for nitrendipine and $3.18 \times 10^{-2} \text{h}^{-1}$ for nisoldipine were obtained. Also, activation energies of 18 kcal/mol and 16 kcal/mol for nitrendipine and nisoldipine, respectively, were calculated. Furthermore, for the nitrendipine hydrolytic degradation test in different solutions for parenteral use were studied. All the tested vehicles significantly increased the degradation of the drug, with the composition of solutions being the most relevant factor rather than the pH.

Keywords: Differential pulse voltammetry, Degradation, 1,4-Dihydropyridines, Hydrolysis

1. Introduction

Nitrendipine and nisoldipine (Fig. 1) are two calcium antagonist drugs belonging to the 1,4-dihydropyridine class, widely used in cardiovascular therapy [1, 2]. Chemically these drugs differ by the position of the nitro group in the aromatic ring and the kind of the ester substituent in position 3. From a pharmacological point of view both the integrity of the chemical structure and the stereochemistry must be retained to develop the optimal interaction with the receptor and thus have the pharmacological and therapeutic efficacy. Consequently, possible hydrolytic changes in the ester groups linked to 1,4-dihydropyridine moiety in both 3 and 5 positions will produce changes in its pharmacological properties [3].

In addition, drug stability is a high impact property affecting both efficacy and toxicity. Generally it is not sufficient to know if the active ingredient concentration corresponds to the amount declared in the pharmaceutical formulation, but it is necessary to know if it exists and or not degraded and the kind of the generated products. Taking into account that the drug degradation in most cases produces minimal chemical changes in the structure; it is necessary to account for them with selective analytical tools for the quantification and/or identification of the degradation products.

Some works have been devoted to stability studies of this kind of drugs and mainly involves the photolytic properties of them, including: a UV derivative spectrophotometric study of the photochemical degradation of nisoldipine [4], photodegradation of inclusion complexes of isradipine with methyl-$eta$-cyclodextrin [5], photostability of nifedipine in powder obtained by crushing tablet [6], and effects of photodegradation of nifedipine on dog erythrocyte membranes [7]. Furthermore, screening techniques have been carried out for several 1,4-dihydropyridine drugs and its daylight degradation products using GC-MS [8] and the chemical stability and pharmacokinetics of amlodipine in rabbits by HPLC-UV [9] were evaluated.

From the electrochemical point of view the 1,4-dihydropyridine drugs have been extensively studied, including its voltammetric behavior [10–13] and the development of electroanalytical methods to be applied to pharmaceutical forms determination [14–16]. In addition, photostability studies by electrochemical methods have been also carried out [16–20].

Up today, literature does not report any systematic electrochemical study about the hydrolytic degradation of 1,4-dihydropyridines. However, a study based on the stability of such compounds in different pH conditions could give basic information about a possible pre-systemic degradation, considering that 1,4-dihydropyridine derivatives are commonly administered by oral route. Furthermore, this type of studies also could be useful knowledge to develop new oral liquid dosage forms.

Consequently, the aim of this work is to develop a new selective procedure to follow degradative reactions specifically applied to hydrolytic degradation of nitrendipine and nisoldipine, making use of an electroanalytical technique that can be an adequate tool to this challenge.

2. Experimental

2.1. Reagents and Drugs

Nitrendipine and nisoldipine (100% chromatographically pure) were obtained from Bayer and Labomed Laboratories.
(Santiago-Chile), respectively. All other reagents employed were of analytical grade.

5% and 10% glucose, Ringer-lactate, glucosaline and physiologic solutions supplied by Sanderson Laboratories (Santiago-Chile) were used as the parenteral pharmaceutical vehicles.

2.2. Buffer Solutions

For the voltammetric technique an ethanol/0.25 M phosphate buffer (20/80) solution adjusted at pH 6.8 with concentrated NaOH solution was used. Degradation trials were carried out in a mixture of ethanol/0.04 M Britton-Robinson buffer solution (30/70), adjusted to desired pH with concentrate NaOH or HCl solutions.

2.3. Voltammetric System

The voltammetric measurement was carried out using a Metrohm 693 VA Processor with 694 Stand and two automatic dosing units, Dosimat 685. The voltammograms and results were exported to a PC by means of the specialised 693 VA-Backup software.

A thermostated cell with Ag/AgCl /KCl 3 M reference electrode, platinum auxiliary and glassy carbon (GC) rotating electrode (Ø = 2 mm, Metrohm) as working electrode was used. To use this electrode it was necessary to activate its surface. The selected treatment was applied previous to each determination and consisted in cycling the applied potential between $E_i = -400$ mV and $E_f = +1800$ mV at 25 V/s in fresh supporting electrolyte for 40 s.

2.4. Repeatability and Reproducibility Studies

For repeatability, ten runs of $2 \times 10^{-5}$ M solution of each drug in ethanol/0.25 M phosphate buffer (20/80) solution at pH 6.8 were assayed by DPV between 400 and 1000 mV. Before each voltammetric run an electrochemical pretreatment was carried out to minimize the electrode adsorption phenomena.

For reproducibility the same procedure as described above was employed but varying the day of assay and the operator.

2.5. Recovery Study

Ten samples independently prepared containing $5.0 \times 10^{-4}$ M solution of each drug was prepared in ethanol/0.04 M Britton-Robinson buffer (30/70). 200 µL from each solution was taken and added to 5 mL of a ethanol/0.25 M phosphate buffer (20/80) solution at pH 6.8 and assayed by DPV between 400 and 1000 mV.

2.6. Measurement Procedure

After performing the electrode pretreatment in 5 mL of fresh electrolyte solution, a 200 µL sample of a sealed vial was added and the potential scanned by DPV between 400 and 1000 mV, each sampling was made in duplicate. This procedure was programmed in the working method of the 693 processor. The quantitation was carried out by means of both a calibration plot and standard addition.

2.7. Degradation Trials

Ethanol/0.04 M Britton-Robinson buffer solutions (30/70) previously adjusted to the desired pH were spiked with nitrendipine or nisoldipine to obtain an initial concentration in the range of $5.0 \times 10^{-4}$ to $9.0 \times 10^{-4}$ M and $1.0 \times 10^{-2}$ to $1.0 \times 10^{-4}$ for each drug, respectively. The solutions were divided over a number of amber vials of 2 mL (two for each point of the degradation curve), sealed hermetically and placed in a oven at 40, 60 or 80.0 ± 0.2 °C. Vials were removed from the oven at selected time intervals, immediately cooled in ice to quench the reaction, and kept in the freezer until DPV analysis when it was necessary.

Degradation was monitored over at least three half-lives. Experiments were carried out in duplicate.

2.8. Degradation in Parenteral Solutions

The same protocol as described above was followed for each drug, but the pH was not adjusted, maintaining the original solution pH. These experiments were carried out at 80 °C.

2.9. Statistic Analysis

Comparison between different techniques, as well as the comparison with standard deviations, was carried out by means of the Student t-test, using significance limits between 95% and 99% of confidence [21, 22].

3. Results and Discussion

The main goal of this work was the development of a new electroanalytic procedure to follow the hydrolytic degradation of two 1,4-dihydropyridine drugs, nitrendipine and nisoldipine. Based on the well-known redox properties of these molecules, a polarographic or an anodic voltammetric method capable of being applied to the hydrolytic kinetic degradation study in different experimental conditions appears as feasible. Thus, these drugs possess two electroactive centers, i.e. nitroaromatic and 1,4-dihydropyridine rings, which can be reduced or oxidized, respectively [10, 23, 24]. Consequently, the use of the above-described electrochemical signals or the appearance of new signals during the time-course of the degradation to quantify this type of phenomena seems to be possible.

First, our results indicated that the polarographic reduction signal do not permit us to follow the time-course of hydrolytic degradation of these drugs. No variation of peak current, or the appearance of new signals was observed. This fact is due to the redox center that undergoes the reduction process is the nitroaromatic moiety [16, 17], and is not affected by the hydrolytic degradation which would occur on the ester groups contained in both the 3- and 5-position of the dihydropyridine structure.

In contrast with the above results, using the oxidation signal, the hydrolytic process could be followed. Thus, following the hydrolysis time-course by DPV, a decrease of the signal
corresponding to the parent drug and a new signal at more negative potentials, were evidenced (Fig. 2). Several variations in both pH and electrolyte supporting composition were carried out, and finally ethanol/0.25 M phosphate buffer (20/80) solution at pH 6.8 was selected as the optimal experimental condition.

Drug absorption on the electrode surface was a critical parameter that affected mainly the reproducibility therefore it was studied. In this sense, a strong adsorption of the analyte on the glassy carbon electrode was observed, which produced a shifting of the drug peak potential at more positive potentials (> 100 mV). This behavior resulted in both a loss of linearity with the increase of concentration and a poor reproducibility of the measurement; therefore electrode pretreatment was necessary. To solve this problem, different treatments described in the literature were tried, including mechanical polishing with alumina [25–27], oxidation with sulphochromic mixture [10,28] and electrochemical treatment [29,30]. From all the treatments tested, the electrochemical treatment produced the most reproducible results.

In Table 1 the analytical assessment of the new voltammetric method for both drugs, nitrendipine and nisoldipine, is summarized. From the analytical parameters obtained it can be concluded that the proposed method fulfills the analytical require with adequate values of repeatability and reproducibility, with coefficient of variations lower than 2%. On the other hand, concentration ranges for calibration plots seem to be adequate to follow degradation with detection limits of 1.5 × 10⁻⁶ M and 2.6 × 10⁻⁶ M and quantitation limits of 4.0 × 10⁻⁶ M and 3.5 × 10⁻⁶ M for nitrendipine and nisoldipine, respectively. The

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nitrendipine</th>
<th>Nisoldipine</th>
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<tbody>
<tr>
<td>Repeatability, CV (%)</td>
<td>1.10</td>
<td>1.68</td>
</tr>
<tr>
<td>Reproducibility, CV (%)</td>
<td>1.56</td>
<td>1.80</td>
</tr>
<tr>
<td>Recovery (%) (CV, %)</td>
<td>98.3 (1.63)</td>
<td>98.5 (1.87)</td>
</tr>
<tr>
<td>Concentration range (M)</td>
<td>5.0 × 10⁻⁶–6.0 × 10⁻⁵</td>
<td>5.0 × 10⁻⁶–6.0 × 10⁻⁵</td>
</tr>
<tr>
<td>Calibration plot $I_p = nA, C = M$</td>
<td>$I_p = 4.061 + 1.327 \times 10^3 C \ (r=0.9993, n = 7)$</td>
<td>$I_p = 27.079 + 1.210 \times 10^7 C \ (r=0.9998, n = 7)$</td>
</tr>
<tr>
<td>Detection limit (M)</td>
<td>1.5 × 10⁻⁶</td>
<td>2.6 × 10⁻⁶</td>
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<td>Quantification limit (M)</td>
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recoveries were higher than 98% with CV of 1.63% and 1.87% for nitrendipine and nisoldipine, respectively. Consequently, the developed voltammetric method is appropriate to follow the hydrolytic kinetic of these 1,4-dihydropyridine drugs (Table 1).

The peak potential values obtained for the parent drugs and their degradation products were 812 ± 6 mV and 665 ± 10 mV for nitrendipine and 778 ± 5 mV and 604 ± 10 mV for nisoldipine, respectively. As can be seen shifting values of 147 mV and 174 mV for nitrendipine and nisoldipine, and their corresponding degradation products, support an adequate selectivity of the proposed method due to that the main signal of each drug is not affected by the appearance of the new signal. Consequently, we can use the voltammetric response of the parent drug to follow the hydrolysis, making possible the kinetic characterization of the degradative process of the drugs.

Finally, the developed voltammetric methodology was applied to follow the hydrolytic degradation kinetic of both 1,4-dihydropyridine drugs. Specifically, we have used the decrease in the voltammetric peak of the 1,4-dihydropyridine parent drugs to follow the kinetics. To test the kinetic order of the hydrolytic degradation, experiments at different initial concentrations and pH were performed. As can be seen from Figure 3, changes in the initial concentration did not affect the slopes of the decay curves. Also, plots of log concentration versus hydrolysis time were

Fig. 4. First order decay plot for: A) nitrendipine and B) nisoldipine hydrolysis in ethanol/0.04 M Britton-Robinson buffer solution (30/70) at 1 × 10^{-3} M concentration, at controlled temperature of 80 °C and pH 12, calculated by DPV experiments in ethanol/0.25 M phosphate buffer (20/80) (pH 6.8) between 400 and 1000 mV.
linear. Consequently, from these experiments we conclude that the hydrolytic degradation of both drugs followed a first order kinetics [3]. In Figure 4 typical decay plots for nisoldipine and nitrendipine in Britton-Robinson buffer at pH 12 and a controlled temperature of 80°C are shown.

In Figure 5 (A and B), the first order decay constants for nisoldipine and nitrendipine in Britton-Robinson buffer at different pHs and at a controlled temperature of 80°C were plotted. As can be seen, from pH > 8 the hydrolytic degradation of both drugs significantly increased with pH, but the magnitude of the calculated constants for both drugs do not differ significantly between them, being nitrendipine slightly more labile than nisoldipine in these conditions.

From the evolution of the calculated decay constants with pH presented in Figure 5 C, it can be concluded that both drugs increased considerably their degradation at strong alkaline pHs and decomposition depends on the concentration of hydroxyl ion. Furthermore, the shape of the apparent kinetic constant plots corresponds to a specific base-catalysis, but general catalysis by buffer composition also can be a contribution. At pH 12 decay constant values of $4.85 \times 10^{-2}\text{h}^{-1}$ for nitrendipine and $3.18 \times 10^{-2}\text{h}^{-1}$ for nisoldipine were obtained.

In Figure 6, the influence of temperature on the degradation of A) $1 \times 10^{-4}\text{M}$ nitrendipine and B) $5 \times 10^{-6}\text{M}$ nisoldipine in ethanol/0.04 M Britton-Robinson buffer solution (30/70), pH 12, determined by DPV in ethanol/0.25 M phosphate buffer (20/80) (pH 6.8) between 400–1000 mV.

Table 2. Influence of different pharmaceutical vehicles on the hydrolytic degradation of nitrendipine at 80 °C calculated by DPV.

<table>
<thead>
<tr>
<th>Formulation [a]</th>
<th>$K(\text{hr}^{-1}) \times 10^7$</th>
<th>$K_{\text{formulation}}/K_{\text{control}}$ [b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ringer-lactate</td>
<td>0.748</td>
<td>41.8</td>
</tr>
<tr>
<td>Physiologic solution</td>
<td>0.686</td>
<td>38.3</td>
</tr>
<tr>
<td>Glucosamine solution</td>
<td>0.962</td>
<td>53.7</td>
</tr>
<tr>
<td>5% Glucose</td>
<td>0.990</td>
<td>55.3</td>
</tr>
<tr>
<td>10% Glucose</td>
<td>0.970</td>
<td>54.2</td>
</tr>
</tbody>
</table>

[a] pH 6.7–7.0; [b] ratio of $k$ nitrendipine in parenteral solution vs. $k$ in Britton-Robinson buffer pH 8 ($k=0.0179 \times 10^{-2}\text{h}^{-1}$)
In order to test possible catalytic effects of well-known parenteral solutions on the hydrolytic degradation of these type of molecules, the stability of nitrendipine in 5 and 10% glucose, Ringer-lactate, glucosamine and physiological solutions were assayed. A comparison between hydrolytic degradation in buffer solutions and in the above-described pharmaceutical vehicles at pH 6–7 (normal pH of these preparations) shows that the latter significantly increased the hydrolytic degradation of nitrendipine, with constant values ranging from 40 to 50 folds of those obtained in Britton-Robinson buffer at the same temperature (80 °C) but at pH 12 (Table 2).

4. Conclusions

In accord with the obtained results, it can be concluded that the developed voltammetric procedure is an adequate analytical tool, which is useful when applied to the study of the hydrolytic degradation kinetics of both drugs. Furthermore, if we consider that the proposed method is based on the decay of the signal corresponding to the dihydropyridine ring, probably this method can be further extended to others compounds of this family such as nifedipine, nimodipine, nicardipine or amlodipine.

5. Acknowledgement

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6. References