Determination of Nitrendipine with β-Cyclodextrin Modified Carbon Paste Electrode

C. Yáñez, *L. J. Núñez-Vergara*, and *J. A. Squella**

Bioelectrochemistry Laboratory, Chemical and Pharmaceutical Sciences Faculty, University of Chile, P.O. Box 233, Santiago, Chile
e-mail: asquella@ciq.uchile.cl

Abstract
Carbon paste electrodes modified with β-cyclodextrin have been investigated for voltammetric determination of nitrendipine. The immobilization of the β-cyclodextrin on the carbon paste leads to a modification of the electrode surface that causes a significant increase in the peak current of the nitrendipine reduction, probably due to formation of an inclusion complex between β-cyclodextrin and nitrendipine. This property was used with analytical purposes by developing a stripping differential pulse voltammetric (SDPV) method to determine nitrendipine.

Keywords: Modified carbon paste electrode, β-Cyclodextrin, Nitrendipine

Cyclodextrins (CDs, cyclic oligopyranose oligomers) are important and widely studied examples of host molecular receptors because of their great affinity for hydrophobic molecules in aqueous media [1–3]. CDs, as known, form inclusion complexes with a great variety of analytes [4].

Many of the potential analytical applications of host-guest systems require immobilization of the host molecule. Thus, several works concerning immobilized thiolated cyclodextrin on gold [5–7] and on silver [8] have been published.

The use of CDs to modify CPE, as far as we know, is a totally new challenge in the field of the modified electrodes. There are few published articles revealing carbon paste electrode modified with β-cyclodextrin [9, 10]. Recently, a review about immobilization of cyclodextrins, complexation abilities and analytical applications have been published [11].

In order to exploit these possibilities further, in this article we investigate the electrochemical behavior of the dihydropyridine drug nitrendipine at modified β-CD carbon paste electrode, mainly focused to demonstrate the interaction between nitrendipine and β-CD immobilized on the electrode and to develop an electroanalytical method to determine this drug.

Nitrendipine is a potent and reliable arterial antihypertensive of the famous calcium antagonist series. Nitrendipine inhibits calcium transport in the slow channels, reducing the peripheral resistance and producing an arterial vasodilation with the consequent pressure diminution. This drug is indicated in the basic treatment of essential arterial hypertension [12] and is widely used all over the world.

The electrochemical reduction of nitrendipine on mercury and glassy carbon electrode in hydroalcoholic solutions has been the subject of voltammetric investigations [13, 14].

According to the previous studies a single irreversible 4-electron reduction peak (I_c) at -782 mV was also observed at CPE (Figure 1A, solid line). The observed irreversible peak is due to the four-electron reduction of the aromatic nitro group as shown in the following equation:

$$R-NO_2 + 4e^- + 4\,H^+ \rightarrow R-NHOH + H_2O \qquad (1)$$

In the reverse sweep, an anodic peak II_a at 298 mV was observed. This corresponds to the two-electron oxidation of the hydroxylamine derivative, which is formed during the first negative-going sweep, to form the nitroso derivative. A new reduction peak, II_c, appearing at -267 mV (Figure 1A), on the second negative going potential scan which is paired with the anodic peak II_a and is thought to be the

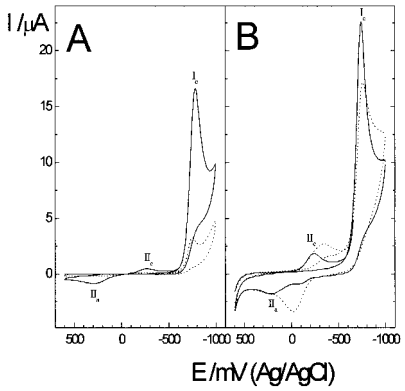

Fig. 1. Cyclic voltammetry at 0.1 V s^{-1} for 1×10^{-4} M nitrendipine in ethanol/0.4 M Britton Robinson: 20/80 at pH 6.0 at CPE (A, solid line) and β-CD modified CPE (B, solid line). Dotted lines show voltammograms after dipping the electrodes into a 1×10^{-4} M nitrendipine solution during 5 minutes and after exchanging the media by only the supporting electrolyte.

reduction of newly formed nitroso compound as shown in the following equation:

$$R - NHOH \leftrightarrow R - NO + 2\,e^- + 2\,H^+ \quad (2)$$

The cyclic voltammograms (CVs) for the reduction of nitrendipine under the same experimental conditions as Figure 1A, at the CD-CPE are shown in Figure 1B (solid line). From the comparison between the CVs at both electrodes we can conclude that the response is slightly different using the CD-CPE. First, the peak current is about 35% higher than that at a carbon paste electrode for peak I_c. Second, the reduction is easier since the cathodic peak potential is approximately 40 mV less negative than the one obtained by using the carbon paste electrode. However, it is clear that the processes (or reactions) on modified carbon paste electrode are the same that at the bare electrode.

However, the difference between the CD-CPE electrode and the bare electrode was evident when submitted to the following experiment. Independently both electrodes were dipped into a 1×10^{-4} M nitrendipine solution during 5 minutes and then each electrode was transferred into a blank supporting electrolyte solution. The cyclic voltammograms obtained from this solution using both CPE and CD-CPE electrodes are shown in Figures 1A and 1B, dotted lines. From this experiment we can distinguish that a very small signal for nitrendipine reduction (peak I_c, dotted line) is observed at the bare CPE. Peaks II_a and II_c are not observed. On the other hand, on CD-CPE, a signal 5-times higher than this obtained by using bare CPE was observed. Moreover, an increase in the current peak II_a and II_c and a change in the peaks potentials are observed.

The above differences in the voltammograms can be ascribed to the immobilization of the β-cyclodextrin on the carbon paste that leads to a modification of the electrode surface that cause a significant increase in the signal due to drug accumulation probably by formation of an inclusion complex nitrendipine – β-CD. This kind of complexes between β-CD and dihydropyridines derivatives (DHP) has been published previously [15–17]. Specifically inclusion complexation of β-CD with nitrendipine has been demonstrated [18], but up to date nobody has used this property from the electrochemical point of view. Due to the cavity size of β-CD (internal diameter of 6.5 Å) and the size of nitrendipine (see Figure 2), the most probable mode of insertion is with the nitro group into the cavity. Moreover

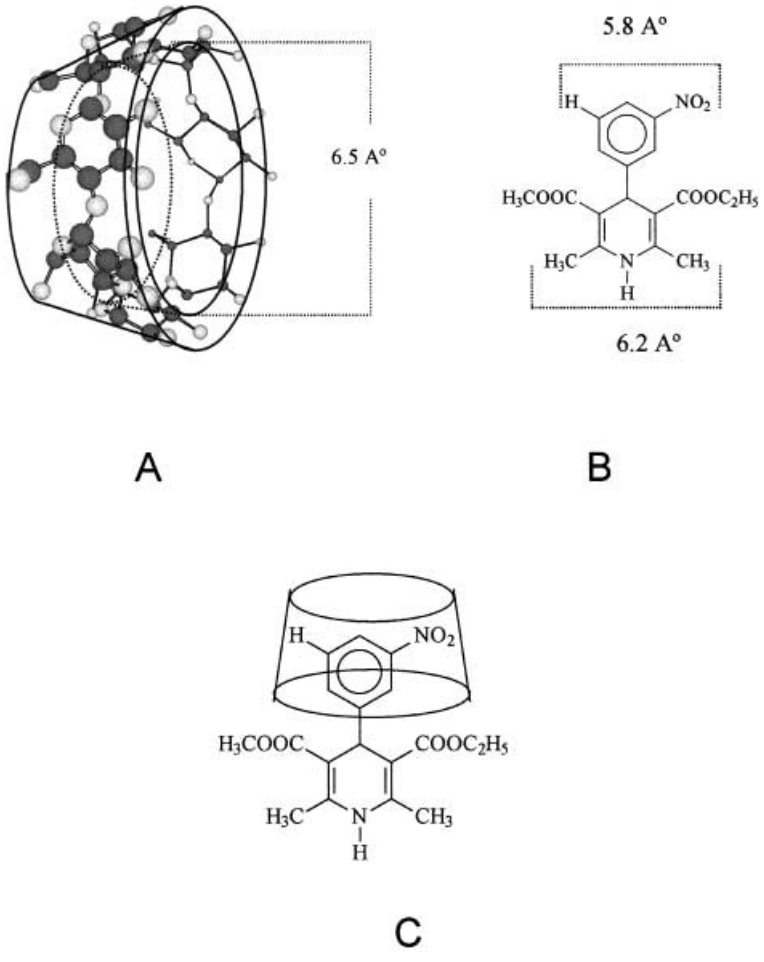

Fig. 2. A) β-Cyclodextrin, toroidal form (6.5 Å internal diameter), B) nitrendipine structure and C) suggested structure of the inclusion complex nitrendipine – β-CD.

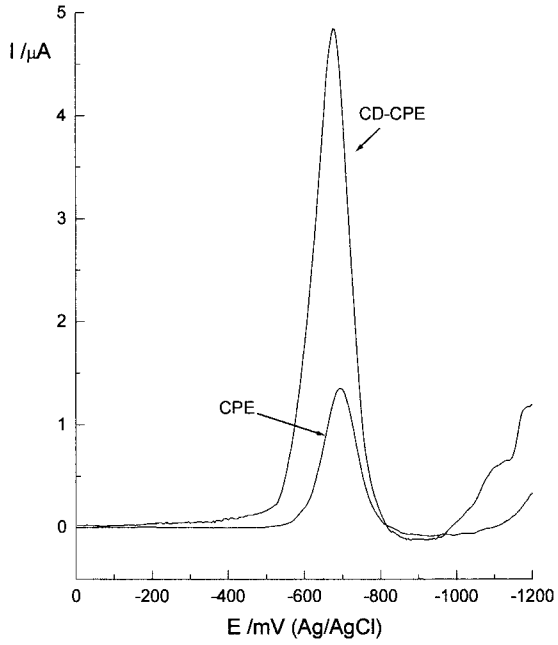

Fig. 3. Stripping differential pulse voltammograms of 1×10^{-5} M nitrendipine in ethanol/0.4 M Britton Robinson: 20/80 at pH 6.0 on β-CD modified CPE and unmodified CPE electrodes. Conditions: 100 s accumulation at 0 V. Pulse amplitude of 50 mV and scan rate of 20 mVs^{-1}

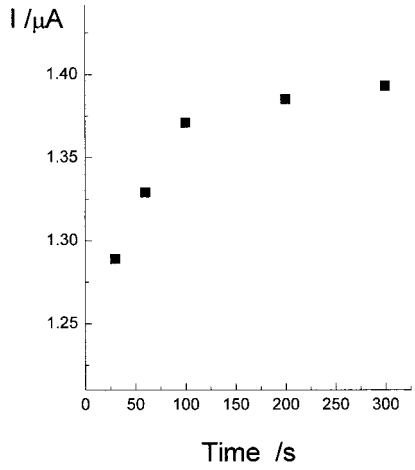

Fig. 4. Effect of the accumulation time on the peak height for 1×10^{-5} M nitrendipine in ethanol/0.4 M Britton Robinson: 20/80 at pH 6.0 on β-CD modified CPE

the fact that the nitroaromatic moiety of the molecule is hydrophobic also supports the postulated mode of insertion.

One of the applications of the above finding can be the improvement of the analytical response of nitrendipine for quantitative determinations. In order to perform an analytical application a stripping differential pulse voltammetric (SDPV) method was tested. Figure 3 shows differential pulse voltammograms of nitrendipine at CPE and CD-CPE, respectively. From these results we can conclude that the peak current increases almost 4-times on CD-CPE compared with CPE. The peak current response depends on the accumulation time reaching saturation at about 100 s (Figure 4). Consequently, an accumulation time of 100 s was chosen for all the measurements. On the other hand we have found that there is no change of the peak current with the accumulation potential in the range -400 to 400 mV. Then, arbitrarily, we chose 0 V as the potential value for polarization of the working electrode.

In order to provide an SDPV quantitative procedure, the dependence between nitrendipine concentration and peak current (i_p) was conducted at both CD-CPE and CPE under the same experimental conditions. For quantitation the calibration plot method, with solutions of nitrendipine in ethanol/0.4 M Britton-Robinson buffer (20/80) (pH 6.0), was used. The calibration plot is described by the following regression curve:

$$I_p\ [\mu A] = 295434.9 \times C\ [M] + 0.743$$
$(r = 0.9925,\ n = 10)$ for CD-CPE

and

$$I_p\ [\mu A] = 147366 \times C\ [M] + 0.160$$
$(r = 0.9946,\ n = 10)$ for CPE

for concentrations ranging between 1×10^{-6} and 1×10^{-5} M.

Where I_p is the peak current and C is the nitrendipine concentration. The repeatability of the measurement was calculated from ten independent runs obtaining a variation coefficient of 3.80% for CD-CPE and 3.43% for CPE. The detection limit was 1.72×10^{-7} M for CD-CPE and 1.3×10^{-6} M for CPE. The quantification limit was 6.7×10^{-7} M for CD-CPE and 3.6×10^{-6} M for CPE and were calculated as is described by Quattrochi [19].

In order to obtain the precision and accuracy of the developed method we have also carried out a recovery study. From this study we have obtained a value of 99.34% for the average recovery with a standard deviation of 3.93 and a coefficient of variation of 3.95%. These results reveal that the proposed method has adequate precision and accuracy and consequently can be validated to the determination of nitrendipine by using β-CD modified carbon paste electrode.

In conclusion, the reported results indicate that β-cyclodextrin immobilized on carbon paste electrode produce a better response compared with unmodified carbon paste electrode by accumulation of nitrendipine. This effect was produced probably by formation of inclusion complex between nitrendipine and β-CD. This method is sensitive enough to be applied to single tablet assay.

Experimental

β-Cyclodextrin was obtained from Fluka and used without further purification. Laboratorio Chile S.A. supplied nitrendipine. 0.4 M Britton-Robinson buffer pH 6.0 solutions were used in all experiments.

Stock solutions of nitrendipine were prepared at a constant concentration of 1×10^{-3} M in ethanol. An aliquot of stock solution was taken and diluted with ethanol/0.4 M Britton-Robinson buffer mixture (20/80) hydroalcoholic solution, to obtain a final working solution concentration between 1×10^{-6} and 1×10^{-4} M. All solutions were shielded from the light to prevent the photodecomposition of nitrendipine.

The working electrode (geometric area 0.126 cm^2) was carbon paste and modified carbon paste with β-cyclodextrin, the counter electrode was a Pt wire, and the reference electrode was Ag/AgCl. All the informed potential values are referred to the Ag/AgCl electrode.

The β-CD modified carbon paste electrode was prepared by mixing 3.0 mL β-CD solution (50 mg in 8.0 mL Britton Robinson buffer pH 6.0) and 500 mg of carbon paste (Metrohm 6.2801.000) in a mortar. The aqueous phase was then allowed to evaporate at about 50 °C. The resulting paste was packed tightly into a Teflon sleeve body. Electrical contact was established with a copper wire. The surface was polished to a smooth finish before use. Then, the stable response of the modified electrodes was obtained through continuous cycling of potentials (10 cycles) at 0.1 V s^{-1} between 0.6 V and − 1.2 V.

Cyclic voltammetric and differential pulse voltammetric experiments were performed with a totally automated BAS 100 voltammetric analyzer connected to a GATEWAY 2000 computer. All the experiments were performed at room temperature. Before all experiments, nitrogen was bubbled through the solution for 10 minutes and was passed above the solution during experiments. Before each electrochemical measurement, the surface of the modified working electrode was mechanically renewed and the application of the continuous cycling of potential was carried out. DPV runs were carried out at pulse amplitude of 50 mV and scan rate of 20 mV s^{-1} after 100 s accumulation at 0 V.

Analytical studies. The repeatability of the measurements was obtained from ten independent runs using 1×10^{-5} M nitrendipine solution in ethanol/0.4 M Britton-Robinson buffer : 20/80 pH 6.0. Both, carbon paste electrode and β-CD modified carbon paste electrode were used. The surface was renewed each time.

For calibration plots, a series of ten solutions containing nitrendipine concentrations between 1×10^{-6} and 1×10^{-5} M in ethanol/0.4 M Britton-Robinson buffer mixture (20/80) at pH 6.0 were prepared.

For recovery studies, a series of ten solutions were independently prepared by weighing approximately 1 mg of nitrendipine and then dissolving in ethanol/0.4 M Britton-Robinson buffer mixture (20/80) at pH 6.0 in order to obtain final concentrations of about 8×10^{-6} M. The mg amount of nitrendipine in the sample solution was calculated from the prepared standard calibration plot.

Acknowledgements

This work was supported by FONDECYT (project Post Doctorate No 3000043 and LC 8000016).

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