Int. J. Electrochem. Sci., 8 **(2013)** 9543 - 9557

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

The Electrochemical Investigation of Inclusion Complexes of Nifedipine and Amlodipine with β-Cyclodextrin and (2-Hydroxypropyl)-β-Cyclodextrin

Z.Z. Stoiljković[1], V.M. Jovanović[2], D.Ž. Mijin[3], V. Nikolić[4], Lj. Nikolić[4], S.D. Petrović[3] and M.L. Avramov Ivić[2]*

[1] Zdravlje, Actavis Company, Leskovac, Vlajkova 199, Leskovac , Serbia
[2] ICTM – Institute of Electrochemistry, University of Belgrade, Njegoševa 12, Belgrade, Serbia
[3] Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, Belgrade, Serbia
[4] Faculty of Technology, Leskovac, University of Nis, Leskovac, Bulevar Oslobodjenja 124, Serbia
[*]E-mail: milka@tmf.bg.ac.rs

Received: 9 May 2013 / *Accepted:* 29 May 2013 / *Published:* 1 July 2013

The electrochemical behavior of inclusion complexes of nifedipine (Nif) and amlodipine (Aml), a long-acting calcium channel blockers dihydropyridine (DHP) class, with β-cyclodextrin (βCD) and (2-hydroxypropyl)-β-cyclodextrin (HPβCD), is examined using cyclic and square wave voltammetry in 0.05 M $NaHCO_3$ and phosphate buffer (pH=11) on a gold electrode. The voltammograms show a single irreversible anodic wave with the current controlled by adsorption. It was found that phosphate buffer favorites the electrochemical activity of both complexes of Nif with the linear dependency of the oxidative currents on their concentrations. In phosphate buffer, only HPβCD-Aml complex showed linear dependency of the oxidative currents on the concentration. In 0.05 M $NaHCO_3$ as electrolyte only HPβCD-Nif exhibited apparent activity. The initial potential of the anodic reaction as well as the value of the potential for anodic currents maximum of all the examined complexes in both electrolytes were shifted to the positive direction compared to their standards. In addition, the value of anodic currents decreased.

Keywords: cyclodextrin complexes; electrochemical determination; gold electrode; cyclic voltammetry; square wave voltammetry

1. INTRODUCTION

Nifedipine, dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate, is a dihydropyridine calcium channel blocker. It is a peripheral and coronary vasodilator, but it has little or no effect on cardiac conduction and negative inotropic activity is rarely seen in therapeutic doses

[1,2]. After oral administration of nifedipine, arterial dilation increases peripheral blood flow, but venous tone does not change [3]. Nifedipine is used in the management of hypertension, angina pectoris, particularly when a vasospastic element is present, as in Prinzmetal's angina, but is not suitable for relief of an acute attack and in the treatment of Raynaud's syndrome [1].

Amlodipine, 2-[(2-aminoethoxy)methyl]4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester, is a second generation 1,4-dihydropyridine derivative of the prototypical molecule nifedipine [2]. Like most of the second generation dihydropyridine derivatives, it has a greater selectivity for the vascular smooth muscle than myocardial tissue, a longer half-life (34 hours), and less negative inotropy than the prototypical nifedipine. Amlodipine is used in the treatment of chronic stable angina and in the management of mild-to-moderate essential hypertension. It is marketed as the benzene sulfonic acid salt (besylate) [1,2,4].

1,4-Dihydropyridine calcium channel antagonist drugs are characterized by a high tendency to degradation when exposed to light. Oxidative aromatization of dihydropyridine fragment to the pyridine moiety is one of the main degradation pathways of amlodipine and related molecules of 1,4-dihydropyridine family (such as nifedipine) and occurs both in solution and in solid state and is promoted by light (Fig. 1) [5-8]. These drugs absorb intensively in the UV-A (some derivates also in the visible) and are known to be photolabile [8-12]. When amlodipine and corresponding besylate were irradiated in a solution, both in the presence and in the absence of air, it was found to give the aromatized pyridine as the main product [12,13]. Under exposition of nifedipine to daylight or to certain wavelengths of artificial light it is converted to a nitrosophenylpyridine derivative, while expose to ultraviolet light leads to formation of nitrophenylpyridine derivative [1,14-16].

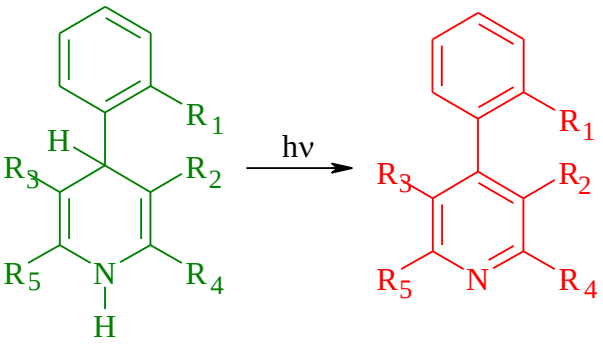

Figure 1. 1,4-Dihydropyridine oxidative degradation to pyridine derivative under the influence of light – amlodipine (R_1=Cl; R_2=COOMe; R_3=COOEt; R_4=$CH_2OCH_2CH_2NH_2$; R_5=Me), nifedipine (R_1=NO_2; R_2,R_3=COOMe; R_4,R_5=Me).

Photo-degradation products of amlodipine and nifedipine do not have pharmacological activity thus prevention of photo-degradation of their formulations is very important. For this purpose different kind of protective agents are used. Several formulations including cyclodextrins, liposomes and microspheres have been prepared and characterized [17-21]. Various organic molecules can be encapsulated in the cyclodextrin cavity, forming so-called inclusion complexes. Among the natural

cyclodextrins, α-, β- and γ-cyclodextrins consisting of 6, 7 and 8 D-glucopyranose units, respectively are the most frequently used for these purpose. The glucosidal units are linked by α-1,4 glycoside links to form the cyclic structures [22,23].

The inclusion complexes (Fig. 2) of nifedipine with β-cyclodextrin [24] (βCD-Nif) or (2-hydroxypropyl)-β-cyclodextrin (HPβCD-Nif) and amlodipine besylate with β-cyclodextrin (βCD-Aml) or (2-hydroxypropyl)-β-cyclodextrin [25] (HPβCD-Aml) were prepared in solid state by co-precipitation with 1:1 mol ratio and characterized by the application of spectroscopic methods FTIR, ^{1}H-NMR and XRD. Formation of inclusion complexes with cyclodextrin alters the physical properties of the included components such as solubility, dissolution rate, photo-sensitivity and stability [17,24-29].

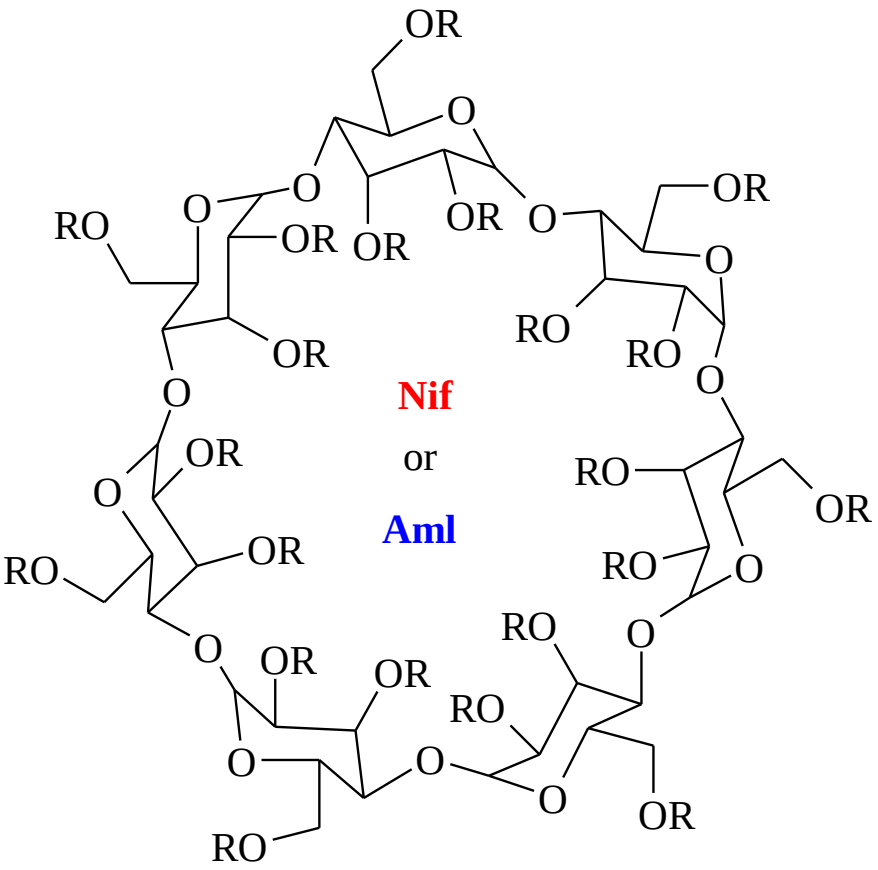

Figure 2. Structure of β-cyclodextrin (βCD) (R=H) and (2-Hydroxypropyl)-β- cyclodextrin (HPβCD) (R=$CH_2CH_2(OH)CH_3$); Nif = nifedipine, Aml = amlodipine

The aim of the work is the investigation of the electrochemical activity of inclusion complexes as well as the possibility of their determination on gold electrode in phosphate buffer (pH=11) and in 0.05 M $NaHCO_3$ using cyclic voltammetry and square wave voltammetry. The changes of the values of the initial oxidative potential as well as of the potential and of the value of anodic currents maximum of inclusion complexes compared to the amlodipine and nifedipine standards were also studied.

2. EXPERIMENTAL

2.1. Materials

Nifedipine and amlodipine besylate (pure substance) were kindly provided by Zdravlje Actavis Company, Leskovac, Serbia. Complexing agents β-cyclodextrin and (2-hydroxypropyl)-β-cyclodextrin of purity 98% and 97.5% were purchased by Merck, Darmstadt and Sigma-Aldrich, Wisconsin, respectively. Potassium dihydrogen phosphate ($KH_2PO_4xH_2O$), and $NaHCO_3$ were p.a. purity, obtained from Merck. Potassium hydroxide was p.a. purity obtained from J. T. Baker. HPLC grade of methanol and acetonitrile were purchased from J.T. Baker. Ammonium acetate and orthophosphoric acid were purchased from Merck. For preparing solution of ammonium acetate purified water was used. Water was purified by Milli-Q system.

2.2. Preparation of standard and stock solutions

Standard stock solution of nifedipine in acetonitrile (c=0.604 mg mL^{-1}) and standard stock solution of amlodipine besylate in mobile phase (c=0.612 mg mL^{-1}) were used. Working solutions of nifedipine and amlodipine besylate were prepared by diluting of stock solutions with acetonitrile or the mobile phase, respectively. These solutions were used to obtain the calibration graph for HPLC analysis.

2.3. Preparation of inclusion complexes by co-precipitation

2.3.1. Inclusion complex formed by nifedipine with cyclodextrin

Nifedipine (1 mmol, 346 mg) and β-cyclodextrin (1 mmol, 1135 mg) or 2-hydroxypropyl-β-cyclodextrin (1 mmol, 1540 mg) were mixed and dissolved in 150 mL of purified water. The light-protected solution was mixed at room temperature for 24 hours, evaporated in a vacuum evaporator at 50 °C to approx. 20 mL volume, and then dried at a temperature 25 °C in a desiccator above concentrated sulphuric acid. After drying the obtained crystalline complexes are used as such for further investigation [24].

2.3.2. Inclusion complex formed by amlodipine besylate with cyclodextrin

Amlodipine besylate (1 mmol, 567 mg) and β-cyclodextrin (1 mmol, 1135mg) or 2-hydroxypropyl-β-cyclodextrin (1 mmol, 1540 mg) were mixed and dissolved in 150 mL of purified water. The light-protected solution was mixed at room temperature for 24 hours, evaporated in a vacuum evaporator at 50 °C to approx. 20 mL volume, and then dried at a temperature 25 °C in a desiccator above concentrated sulphuric acid. After drying the obtained crystalline complexes are used as such for further investigation [25].

The whole process is performed in darkness in order to protect nifedipine or amlodipine besylate from photodegradation. The molar ratio in the formed complexes of nifedipine or amlodipine besylate with complexing agents (β-cyclodextrin or 2-hydroxypropyl-β-cyclodextrin) is 1:1.

2.4. Cyclic voltammetry

Standard equipment was used for the cyclic voltammetry (CV) measurements and the three electrode electrochemical cell was described in detail previously [30].

Polycrystalline gold served as the working electrode, a gold wire was used as the counter electrode and a saturated calomel electrode as the reference electrode. Polycrystalline gold (Pine rotating disc, used as stationary electrode, surface area 0.500 cm^2) was polished with diamond paste, cleaned with a mixture of purified water and sulfuric acid and further cleaned with purified water in an ultrasonic bath. All the potentials are given *vs*. SCE. Prior to the addition of cyclodextrin complexes the electrolyte was deoxygenated by purging with nitrogen. All the experiments were performed at room temperature. After the addition of inclusion complexes, only the first sweep is recorded and the electrode is polished and cleaned between the two consecutive investigated concentrations.

2.5. Square wave voltammetry

Square wave voltammetry (SWV) measurements were performed using AUTOLAB potentiostat/galvanostat (Metrohm, ECO Chemie, The Netherlands). The operating parameters were: potential step of 2 mV, pulse of 20 mV, frequency 8 Hz and scan rate 15 mV s^{-1}.

2.6. Preparation of the standard solutions for the analysis of complexes

Appropriate amount of inclusion complexes was weight and dissolved in purified water or purified water-methanol mixture 1:1. Complexes with β-cyclodextrin were dissolved in warm water while complexes with 2-hydroxypropyl-β-cyclodextrin were dissolved at room temperature. For complexes with nifedipine water-methanol mixture 1:1 were used.

2.7. Equipment and chromatographic conditions

2.7.1. Apparatus

HPLC system (Agilent 1100 Series) equipped with binary pump, Diode Array Detector, column thermostat and the thermostatted autosampler was used. ChemStation 32 software was used for the data acquisition.

2.7.2. HPLC analysis of nifedipine-cyclodextrin complexes

Hypersil ODS column (Agilent, 250.0 mm x 4.6 mm id, 5 μm particle size) and the mobile phase consisted of acetonitrile and 0.03% v/v solution of orthophosphoric acid (40:60, v/v) was used. pH of mobile phase was 2.67. The flow rate was maintained at 1.0 mL/min. The mobile phase was passed through 0.45 μm membrane filter (Sartorius Stedim Biotech GmbH, Goettingem, Germany) and degassed before use. The elution was monitored with diode array detector at 235 nm. The injection volume was 20 μL. The test was carried out protected from light. All the chromatographic separations were carried out at controlled room temperature (20-25 °C).

2.7.3. HPLC analysis of amlodipine besylate-cyclodextrin complexes

The Spherisorb ODS1 (octadecylsilyl silica gel) column (Waters, 250.0 mm x 4.0 mm id, 5 μm particle size) and the mobile phase consisting of 2.3 g/L ammonium acetate solution - methanol (30:70, v/v) was used. The flow rate was maintained at 1.0 mL min^{-1}. The mobile phase was passed through 0.45 μm membrane filter (Sartorius Stedim Biotech GmbH, Goettingem, Germany) and degassed before use. The elution was monitored with diode array detector at 237 nm. The injection volume was 20 μL. The test was carried out protected from light. All the chromatographic separations were carried out at controlled room temperature (20-25 °C).

3. RESULTS AND DISCUSSION

The oxidative behavior of inclusion complexes of HPβCD-Aml, βCD-Aml, HPβCD-Nif and βCD-Nif was studied in the same way as for amlodipine standard determination [31].

3.1. Cyclic voltammetry and square wave voltammetry of cyclodextrin complexes with nifedipine in phosphate buffer

Cyclic voltammograms of βCD-Nif are presented in Fig. 3 in phosphate buffer (pH=11) showing that in anodic direction its electrooxidation begins at 0.25 V with the increasing tendency until the current maximum appearing at the beginning of the oxide formation on gold electrode. This maximum current value appears in the whole range of the oxide formation with slightly increasing from 0.9 V to 1.0 V. In cathodic direction the presence of βCD-Nif leads to the smaller currents of the oxide reduction which is attributed to the reduction of the species oxidized in anodic direction.

The linear dependency of anodic currents *vs*. concentration of nifedipine in βCD-Nif in a range: 2.24 – 5.53 μg mL^{-1} was obtained at 0.77 V from the data in Fig. 3. The mentioned linear relationship corresponds to the equation 1 given in Table 1 [32].

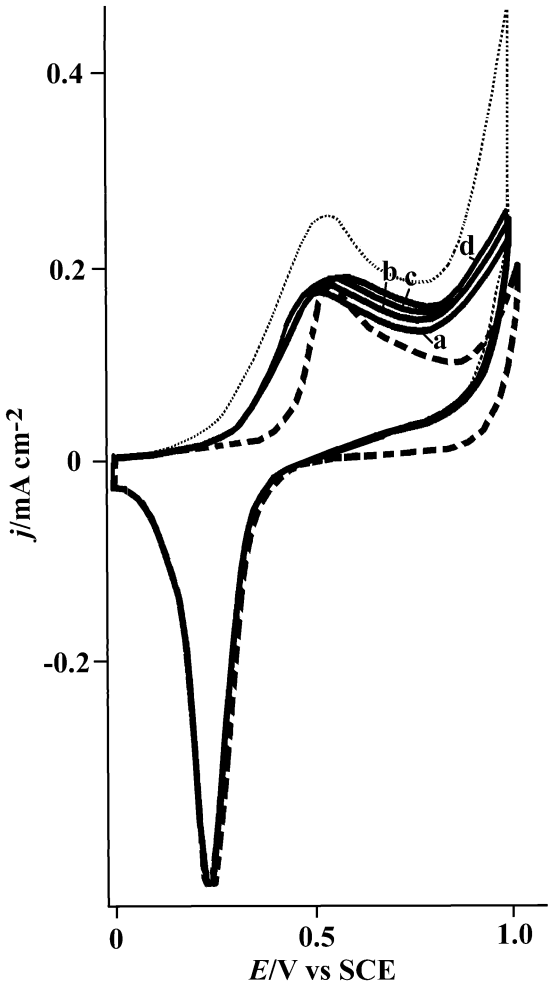

Figure 3. Cyclic voltammogram of gold electrode in phosphate buffer (pH=11) (dashed line) and in a presence of βCD-Nif. Nifedipine concentration: a) 2.24 μg mL^{-1}, b) 3.35 μg mL^{-1}, c) 4.44 μg mL^{-1}, d) 5.53 μg mL^{-1}, sweep: 50 mV s^{-1}; nifedipine standard 2.7 μg mL^{-1} (dotted line).

Table 1. The linear dependency of anodic currents *vs*. concentration of nifedipine and amlodipine in studied complexes

No.	Complex	Buffer	Method	Equation, j/mA cm^{-2}=$f(C$/μg mL^{-1})	R
1	βN[a]	Ph[d]	CV	j =0.1226 (±0.0025)+0.0098 (±0.0006) C	0.9962
2	βN	Ph	SWV	j_{pa}=0.0876 (±0.0003) + 0.0019 (±0.00004) C	0.9998
3	HN[b]	Ph	CV	j=0.1453 (±0.0018)+0.0258 (±0.0006) C	0.9996
4	HN	Ph	SWV	j_{pa}=0. 0727 (±0.0039) + 0.0069 (±0.0014) C	0.9802
5	HN	SBC[e]	CV	j=0.0782 (±0.0021)+0.0333 (±0.0011) C	0.9995
6	HN	SBC	SWV	j_{pa}=0.0706 (±0.0004)+0.0021 (±0.0001) C	0.9986
7	HA[c]	Ph	CV	j=0.0679 (±0.0030)+0.0132 (±0.0004) C	0.9994
8	HA	Ph	SWV	j=0.0353 (±0.0008)+0.0006 (±0.00006) C	0.9885

[a] βCD-Nif; [b] HPβCD-Nif; [c] HPβCD-Aml; [d] phosphate buffer; [e] 0.05 M $NaHCO_3$

Square wave voltammetry, as a fast, sensitive technique with low detection limit, was used for quantitative determination of nifedipine in βCD-Nif on the gold electrode. The square wave anodic stripping voltammograms for different concentrations of nifedipine in βCD-Nif, recorded in phosphate

buffer in the potential range from 0 to 1.0 V at the scan rate of 15 mV s^{-1} are presented in Fig. 4. Before each scan, the compound was accumulated at the electrode surface at 0.1 V during 180 s. Each voltammogram is characterized by the well defined peak at approximately 0.45 V and it is attributed to the oxidation of adsorbed inclusion complex. The current of anodic stripping peak exhibits linear dependence on the βCD-Nif concentration.

The linear dependency of anodic peak currents *vs*. concentration of nifedipine in βCD-Nif in a range: 4.44 – 8.72 μg mL^{-1} was obtained from the data in Fig. 4. The linear relationship is given by the equation 2 given in Table 1.

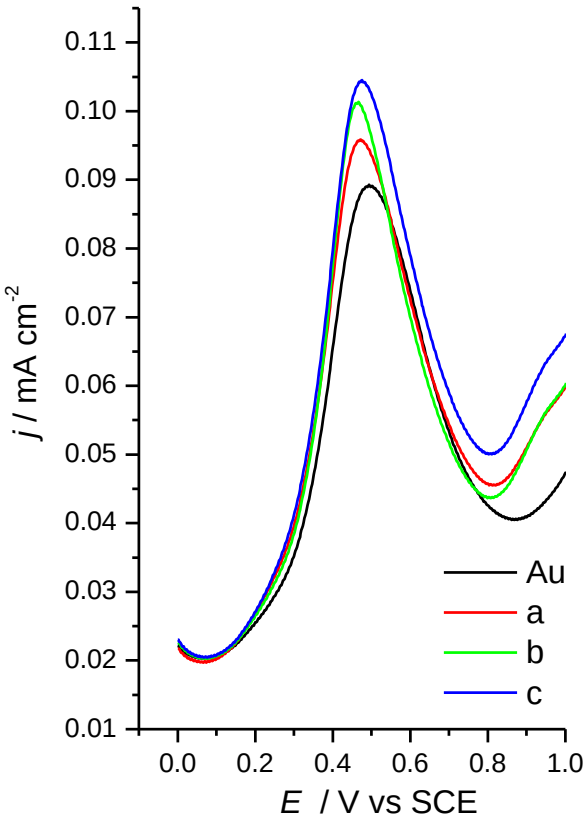

Figure 4. Square wave anodic stripping voltammograms at gold electrode in phosphate buffer (pH=11) and in a presence of βCD-Nif. Nifedipine concentration: a) 4.44 μg mL^{-1}, b) 6.66 μg mL^{-1}, c) 8.72 μg mL^{-1}. Accumulation time: 220 s at E = 0.1 V; step size 2 mV, pulse size 20 mV, frequency 8 Hz, scan rate 15 mV s^{-1}.

Cyclic voltammograms of HPβCD-Nif presented in Fig. 5 in phosphate buffer (pH=11) show the same electrochemical behavior as was observed for βCD-Nif (Fig. 3). The linear dependency of anodic currents *vs*. concentration of nifedipine in HPβCD-Nif in a range: 1.82 – 3.60 μg mL^{-1} was obtained at 0.65 V from the data given in Fig. 5. The linear relationship corresponds to the equation 3 (Table 1).

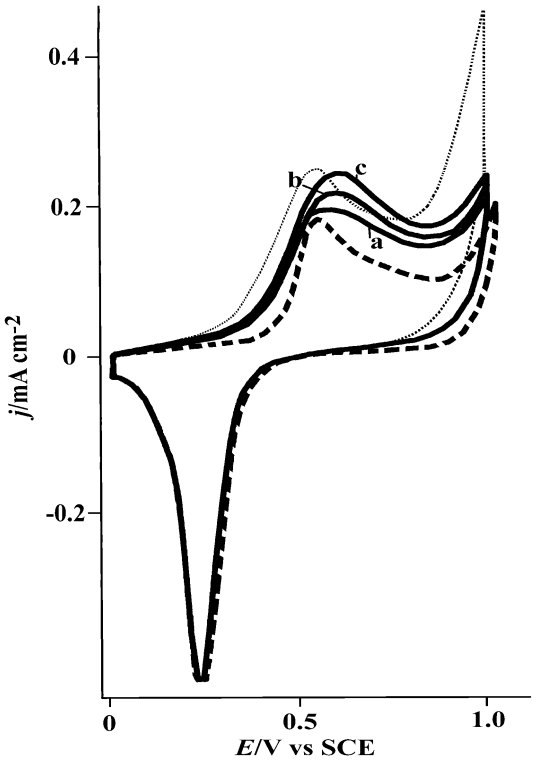

Figure 5. Cyclic voltammogram of gold electrode in phosphate buffer (pH=11) (dashed line) and in a presence of HPβCD-Nif. Nifedipine concentration: a) 1.82 μg mL^{-1}, b) 2.71 μg mL^{-1}, c) 3.60 μg mL^{-1}, sweep: 50 mV s^{-1}; nifedipine standard 2.7 μg mL^{-1} (dotted line).

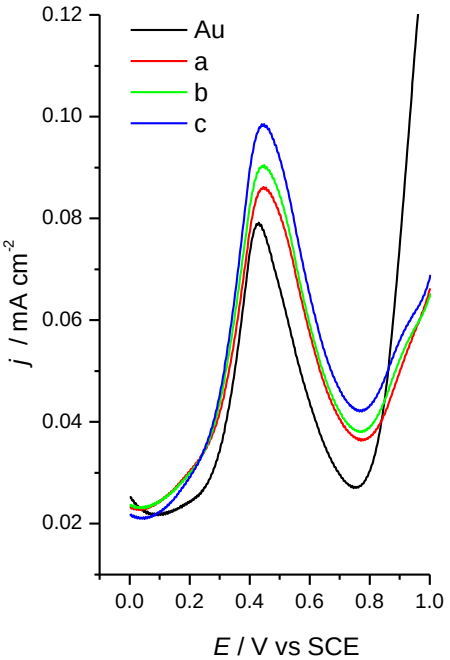

Figure 6. Square wave anodic stripping voltammograms at gold electrode in phosphate buffer (pH=11) and in a presence of HPβCD-Nif. Nifedipine concentration: a) 1.82 μg mL^{-1}, b) 2.71 μg mL^{-1}, c) 3.60 μg mL^{-1}. Accumulation time: 180 s at E = 0.1 V; step size 2 mV, pulse size 20 mV, frequency 8 Hz, scan rate 15 mV s^{-1}.

Quantitative determination of nifedipine in HPβCD-Nif on the gold electrode was also performed by square wave voltammetry. SWV of HPβCD-Nif showed similar behavior as βCD-Nif under the same experimental conditions. The results are presented in Fig. 6. Again, each voltammogram is characterized by the well defined peak at approximately 0.45 V and it is attributed to the oxidation of adsorbed inclusion complex. The current of anodic stripping peak exhibits linear dependence on the HPβCD-Nif concentration in a range: 1.82 – 3.60 μg mL^{-1}. The mentioned linear relationship corresponds to the equation 4 (Table 1).

3.2. Cyclic voltammetry and square wave voltammetry of cyclodextrin complexes with nifedipine in 0.05 M $NaHCO_3$

Although, we have showed earlier [31] that the phosphate buffer is more suitable for the anodic oxidation of dihydropyridine class drugs, we tested 0.05 M $NaHCO_3$ as electrolyte as well. All the examined inclusion complexes exhibited apparently lower electrochemical activity than in phosphate buffer. The lower activity but good linearity of the currents *vs*. concentrations was observed only in the case of the HPβCD-Nif. The linear dependency of anodic currents *vs*. its concentrations appears at 300 mV more positive potential than was the case in phosphate buffer (Figs. 5 and 7). The linear dependency in a range: 0.91 – 2.71 μg mL^{-1} obtained at 0.95 V from the data in Fig. 5 is given in Table 1 by the equation 5.

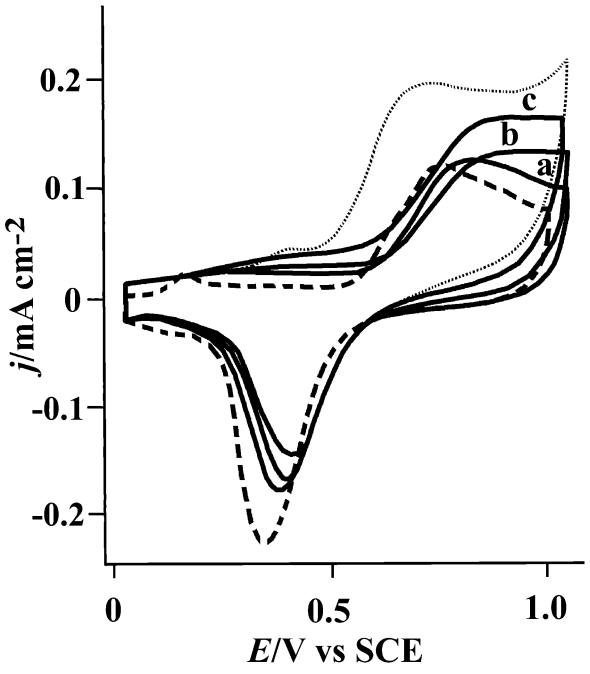

Figure 7. Cyclic voltammogram of gold electrode in 0.05 M $NaHCO_3$ (dashed line) and in a presence of HPβCD-Nif (full line). Nifedipine concentration: a) 0.91 μg mL^{-1}, b) 1.82 μg mL^{-1}, c) 2.71 μg mL^{-1}, sweep: 50 mV s^{-1}; nifedipine standard 2.7 μg mL^{-1} (dotted line).

Square wave voltammetry showed also the lower electrochemical activity of nifedipine in HPβCD-Nif in 0.05 M $NaHCO_3$ (lower anodic currents) (Fig. 8) than in phosphate buffer (Fig. 6). Each

voltammogram is characterized by the well defined peak at approximately 0.6 V which is more than 100 mV shifted to the positive potential comparing to phosphate buffer. The linear dependency of peak currents *vs.* concentration of nifedipine in HPβCD-Nif in a range: 0.91 – 2.71 μg mL^{-1} is presented by the equation 6 (Table 1).

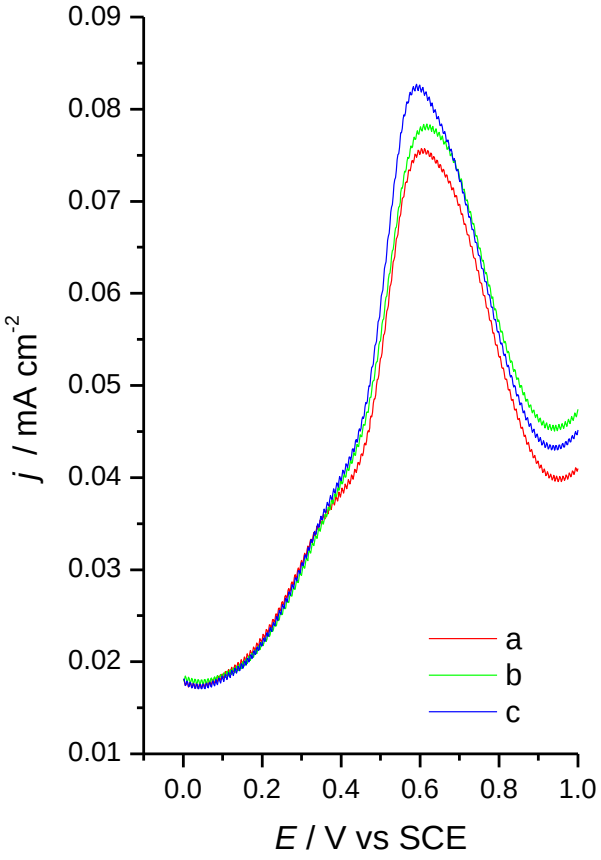

Figure 8. Square wave anodic stripping voltammograms at gold electrode in 0.05 M $NaHCO_3$ and in a presence of HPβCD-Nif. Nifedipine concentration: a) 0.91 μg mL^{-1}, b) 1.82 □ μg mL^{-1}, c) 2.71 μg mL^{-1}. Accumulation time: 180 s at E = 0.1 V; step size 2 mV, pulse size 20 mV, frequency 8 Hz, scan rate 15 mV s^{-1}.

3.3. Cyclic voltammetry and square wave voltammetry of cyclodextrin complexes with amlodipine in phosphate buffer

In the form of inclusion complexes amlodipine exhibited much lower electrochemical activity in both electrolytes compared to nifedipine complexes. Only HPβCD-Aml in phosphate buffer exhibited one apparent electrochemical activity as is presented in Fig. 9. Cyclic voltammograms show an increase in anodic activity in the whole area of the oxide formation on gold electrode with the linear dependency on the concentrations at 0.85 V. The linear dependency of peak currents *vs.* concentration of amlodipine in HPβCD-Aml in a range: 3.26 – 9.58 μg mL^{-1} is presented by the equation 7 (Table 1).

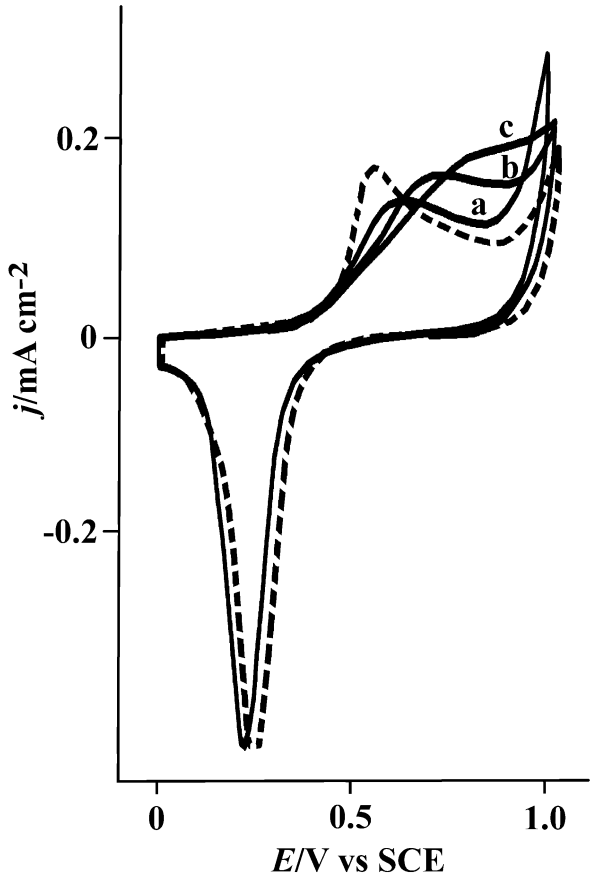

Figure 9. Cyclic voltammogram of gold electrode in phosphate buffer (pH=11) (dashed line) and in a presence of HPβCD-Aml. Amlodipine concentration: a) 3.26 μg mL^{-1}, b) 6.45 μg mL^{-1}, c) 9.58 μg mL^{-1}, sweep: 50 mV s^{-1}.

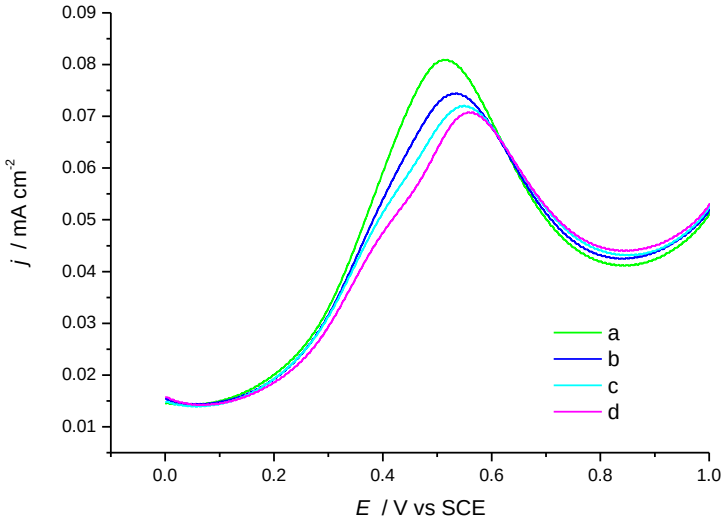

Figure 10. Square wave anodic stripping voltammograms at gold electrode in phosphate buffer (pH=11) and in a presence of HPβCD-Aml. Amlodipine concentration: a) 9.58 μg mL^{-1}, b) 11.13 μg mL^{-1}, c) 12.65 μg mL^{-1}, d) 14.17 μg mL^{-1}. Accumulation time: 180 s at E = 0.1 V; step size 2 mV, pulse size 20 mV, frequency 8 Hz, scan rate 15 mV/s.

Square wave voltammetry showed the apparent electrochemical activity of amlodipine in HPβCD-Aml in the phosphate buffer (Fig. 10). As cyclic voltammograms show, the linear dependency was recorded at 0.85 V. The linear dependency in a range: 9.58 – 14.17 μg mL^{-1} is presented by the equation 8 (Table 1).The values of the all concentrations of nifedipine and amlodipine in studied complexes (by CV and SWV) as is presented in Tab 1 are confirmed by HPLC method.

The characterization of cyclodextrin (CD) systems by electrochemical methods, mainly by cyclic voltammetry has been discussed [33-36] with the opinion that the presence of CD in the electrolyte solution causes a decrease in the peak current and also a shift in the apparent half-wave potential in cyclic voltammetry.

Comparing to the electrochemical behavior of Aml [31] and Nif standards under the identical experimental conditions it was observed for all the examinated complexes that the complexation causes two main changes in cyclic voltammograms in both electrolytes. For example, this can be shown by comparison of the HPβCD-Nif (full line) and Nif standard (dotted line) (Figs. 5 and 7). Firstly, the initial potential of the anodic reaction is shifted to the positive direction, the value of the potential for the obtained linearity of the anodic currents *vs.* concentrations is also shifted to the positive direction and secondly, the value of anodic currents decreased. These changes in all the discussed inclusion complexes [33-36] are ascribed to the inclusion complexes formed and in a case presented in Fig. 7, to the formed HPβCD-Nif.

The change in the initial potential reveals that amlodipine and nifedipine molecules bonded in complexes were oxidized with more difficulty. For example, in a case of indapamide and its complexation with β-cyclodextrin anodic peak was also shifted to the more positive potential comparing to indapamid and anodic currents decreased [37]. The same effect (the potential shift to the more negative value during the electroreduction and the currents decrease was observed for the inclusion complex of β-cyclodextrin and furnidipine [38]. A decrease in the electrochemical response was also observed during in-vivo evaluation of the controlled release effects of the mediator- β-cyclodextrin complexes to microbial delivery [39].

By comparing the chemical structures of amlodipine and nifedipine, it is evident that presence of NO_2 group with a strong hydrophobic effect (coefficient hydrophobic substituent, π=-0.85 [40]) significantly decrease the total hydrophobic characteristics of nifedipine. On the other hand, the presence of Cl group as substituent (π=+0.36 [40]) increase the hydrophobic properties of amlodipine. It is important to note that the contribution of the hydrophobic effect to drug/CD complex stability is evident and significant but varies with the structure of drug species [41]. These facts are in good agreements with our experimental data that HPβCD-Aml complex is more subjected to electrochemical adsorption and accumulation.

4. CONCLUSION

It was shown by cyclic voltammetry and square wave voltammetry that phosphate buffer favorites the electrochemical activity of inclusion complexes of nifedipine and an apparent electrochemical activity and the linear dependency of the oxidative currents on concentrations of

HPβCD-Nif and βCD-Nif was found. The electrochemical activity of nifedipine incorporated in complexes is independent of the type of cyclodextrin complex.

By testing 0.05 M $NaHCO_3$ as electrolyte, nifedipine inclusion complexes exhibited much lower electrochemical activity than in phosphate buffer. The good linearity of the currents versus concentrations is observed only in the case of the HPβCD-Nif but with the two times lower currents in the area of the oxide formation.

The phosphate buffer favorites only HPβCD-Aml, with the linear dependency of the oxidative currents on concentrations. By testing 0.05 M $NaHCO_3$ as electrolyte amlodipine inclusion complexes exhibited the low activity as well as the absence of the linearity of the anodic currents *vs*.concentrations.

It was observed that complexation with amlodipine and nifedipine in all the cases causes two main changes in cyclic voltammograms compared to their standards. Firstly, the initial potential of the anodic reaction as well as the value of the potential for anodic currents maximum is shifted to the positive direction. Secondly, the value of anodic currents decreases. These effects are attributed to the inclusion complexes formed and confirmed their successful formation.

ACKNOWLEDGEMENT
The authors acknowledge the financial support by the Ministry of Education and Science of the Republic Serbia (Project Number 172013).

References

1. *Martindale The Complete Drug Reference*, 36th ed., The Pharmaceutical Press, London (2009), pp. 1217, 1089
2. J.M. Beale Jr and J.H. Block, *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry*, 12th ed., Lippincott Williams and Wilkins, Philadelphia (2011) pp. 625, 626
3. *Goodman and Gilman's Manual of Pharmacology and Therapeutics*, Ed. J.G. Hardman, L.E Limbird and A.G. Gilman, The McGraw-Hill Companies, Inc., New York (2008), p. 856
4. USPI Norvasc: Highlights of Prescribing Information, October 2011, www.pfizer.com/products [accessed 29 August 2012]
5. G. Ananchenko, J. Novakovic and J. Lewis, *Profiles of Drug Substances, Excipients, and Related Methodology*, Ed. H.G. Brittain, Elsevier Inc. 37 (2012) 31
6. M.C. Nahata, R.S. Morosco and T.F. Hipple, *J. Am. Pharm. Assoc*., 39 (1999) 375
7. A. Abdoh, M.M. Al-Omari, A.A. Badwan and A.M.Y. Jaber, *Pharm. Dev. Technol*., 9 (2004) 15
8. P.K.F. Yeung, S.J. Mosher and P.T. Pollak, *J. Pharm. Biomed. Anal.,* 9 (1991) 565
9. G.S. Sadana and A.B. Ghogare, *J. Pharm. Sci.,* 80 (1991) 895
10. V. Marinković, D. Agbaba, K. Karljiković-Rajić, S. Vladimirov and J. Nedeljković, *J. Pharm. Biomed. Anal.,* 32 (2003) 929
11. V.D. Marinković, D. Agbaba, K. Karljiković-Rajić, J. Comor and D. Živanov-Stakić, *Farmaco,* 55 (2000) 128
12. G. Ragno, A. Garofalo and C. Vetuschi, *J. Pharm. Biomed. Anal*., 27 (2002) 19
13. E. Fasani, A. Albini and S. Gemme, *Int. J. Phar.,* 352 (2008) 197
14. I.A. Majeed, W.J. Murray, D.W. Newton, S. Othman and W.A. Alturk, *J. Pharm. Pharmacol*., 39 (1987) 1044

15. Y. Mastuda, R. Teraoka and I. Sugimoto, *Int. J. Pharm*., 54 (1989) 211
16. I. Matsuura, M. Imaizumi and M. Sugiyama, *Chem. Pharm. Bull*., 38 (1990) 1692
17. G. Ragno, E. Cione, A. Garofalo, G. Genchi, G. Ioele, A. Risoli and A. Spagnoletta, *Int. J. Pharm.*, 265 (2003) 125
18. M. Aujla, A.C. Rana, N. Seth and R. Bala, *Journal of Drug Delivery and Therapeutics*, 2 (2012) 55
19. L. Zhao, Y-M. Wei, Y. Yu and W-W. Zheng, *Arch. Pharm. Res.*, 33 (2010) 443
20. N. Li, D.S. Kommireddy, Y. Lvov, W. Liebenberg, L.R. Tiedt and M.M. De Villiers, *J. Nanosci. Nanotechno*., 6 (2006) 3252
21. N. Li, M.D. Degennaro, W. Liebenberg, L.R. Tiedt, A.S. Zahn, M.V. Pishko and M.M. De Villiers, *Pharmazie*, 61 (2006) 595
22. J. Szejtli, *Chem. Rev*. 98 (1998) 1743
23. P. Giastas, K. Yannakopoulou and I.M. Mavridis, *Acta Cryst*., B59 (2003) 287
24. V. Nikolić, D. Ilić, Lj. Nikolić, M. Stanković, M. Cakić, Lj. Stanojević, A. Kapor and M. Popsavin, *Cent. Eur. J. Chem*., 8 (2010) 744
25. A. Kapor, V. Nikolić, Lj. Nikolić, M. Stanković, M. Cakić, Lj. Stanojević and D. Ilić, *Cent. Eur. J. Chem*., 8 (2010) 834
26. K.P.R. Chowdary and G.K. Reddy, *Indian Journal of Pharmaceutical Sciences*, 64 (2002) 142
27. M. Bećirević-Laćan, J. Filipović-Grčić, N. Skalko and J. Jalsenjak, *Drug Dev. Ind. Pharm.*, 22 (1996) 1231
28. J. Mielcarek, *J. Pharm. Biomed. Anal*., 15 (1997) 681
29. J. Mielcarek, *Acta Pol. Pharm.*, 52 (1995) 459
30. K.M. Drljević-Djurić, V.D. Jović, U.Č. Lačnjevac, M.L. Avramov Ivić, S.D. Petrović, D. Ž. Mijin and S. B. Djordjević, *Electrochim. Acta*, 56 (2010) 47
31. Z.Z. Stoiljković, M.L. Avramov Ivić, S.D. Petrović, D.Ž. Mijin, S.I. Stevanović, U.Č. Lačnjevac and A.D. Marinković, *Int. J. Electrochem. Sci.*, 7 (2012) 2288
32. R. Greaf, R. Peat, R. L. Peter, D. Pletcher and J. Robinson, *Instrumental Methods in Electrochemistry*, Ellis Horwood, Chichester (1985) p. 178
33. A. Ferancová and J. Labuda, *Anal. Bioanal. Chem*., 370 (2001) 1
34. A. Ferancová, J. Labuda, J. Barek and J. Zima, *Chem. Listy*, 96 (2002) 856
35. P.M. Bersier, J. Bersier and B. Klingert, *Electroanalysis*, 3 (1990) 443
36. A-E. Radi and S. Eissa, *The Open Chemical and Biomedical Methods Journal*, 3 (2010) 74
37. A-E. Radi and S. Eissa, *J. Incl. Phenom. Macrocycl. Chem.*, 71 (2011) 95
38. C. Yanez, R. Salazar, Lj. Nunez-Vergara and J.A. Squella, *J. Pharm. Biomed. Anal.*, 35 (2004) 51
39. Q. Lu, J. Zhao, M. Wang and Z. Wang, *Int. J. Electrochem. Sci.*, 6 (2011) 3868
40. C. Hansch and A. Leo, *Exploring QSAR: Hydrophobic, Electronic and Steric Constants*, ACS, Washington DC (1995) pp. 219-222
41. M.M. Al Omari, M.I. El-Barghouthi, M.B. Zughul, J.E.D. Davies and A.A. Badwan, *J. Mol. Liq.*, 155 (2010) 103