

THE ELECTROCHEMICAL BEHAVIOR OF SOME BETA-BLOCKERS ON SCREEN PRINTED ELECTRODES MODIFIED WITH CALIXARENE

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Abstract

The aim of this work was to study the electrochemical behavior of metoprolol succinate, atenolol and propranolol hydrochloride, respectively.

The first part of the cyclic voltamperometric studies was performed using classical platinum, gold and vitreous carbon electrodes, aiming the electrochemical behavior of beta-blockers at different pH values (5.22-8.59).

The second part of the studies was performed using gold electrodes modified with calixarenes. In the case of using modified electrodes with calixarenes, compared to the unmodified ones, it was observed that the redox process has decrease, due to an interaction between the calixarenes and beta-blocker. This interaction is sustained also by the performed computational chemistry calculations.

Rezumat

Obiectivul acestei lucrări a fost studiul comportamentului electrochimic al metoprololului succinat, atenololului și respectiv propranololului clorhidrat.

Prima parte a studiilor de voltamperometrie ciclică s-au efectuat cu ajutorul electrozilor clasici de platină, aur și carbon vitros, urmărindu-se comportamentul beta-blocantelor la diferite valori ale pH-ului (5,22-8,59).

A doua parte a studiilor a fost efectuată cu ajutorul unor electrozi din aur modificați cu calixarene. S-a observat că intensitatea proceselor redox scade, dacă s-au folosit electrozi modificați cu calixarenă comparativ cu cei nemodificați, datorită existenței unei interacțiuni între calixarenă și beta-blocant. Această interacțiune este susținută și de calculele de chimie computațională, care s-au efectuat.

Keywords: calixarenes, beta-blockers, screen printed electrodes, cyclic voltammetry

Introduction

Beta-blockers are antagonists of beta-adrenergic receptors in the sympathetic nerve endings. By preventing the stimulation of the receptors by the endogenous catecholamines, these medicines have an inhibitory effect upon the physiological properties of the myocardium decreasing its contraction force, its frequency and its excitability. Consequently, beta-blockers are widely used in treating arterial hypertension, *angina pectoris* and cardiac arrhythmia [1].

Given the important therapeutic function of this pharmacological category, various studies have been conducted on the properties, detection and efficiency of beta-blockers.

There are many validated analytical methods for determining beta-blockers in different stages of their use either in qualitative or quantitative control or in clinical studies. Gas chromatography combined with mass spectrometry (GC/MS) or liquid chromatography combined with mass spectrometry (LC/MS) are widely used for detecting metoprolol, atenolol and propranolol. However, the most commonly used method is high performance liquid chromatography (HPLC) combined with different methods of detection such as UV or MS. Chromatographic methods are generally complicated, expensive and laborious. Moreover, because of the numerous derivatizations in the pre-treatment stage, the time necessary for the analysis increases a lot [1,7]. Electrochemical methods play an essential role in the analysis of medicines, as they are sensitive, selective, low cost, and because of the fact that more often than not the excipients do not interfere in the analysis, the sample can be prepared by simply dissolving the substance in a proper solvent. There are also techniques for detecting beta-blockers which combine HPLC and voltamperometric methods [6].

Due to their many advantages, screen printed electrodes (SPE) have primarily been used during the last decade in biomedical analysis, pharmaceuticals and in analyzing environmental samples and foods thus gradually shifting from the technology based on mercury electrode or other classic solid electrodes. These electrochemical sensors became increasingly used for detecting different ionic and/or molecular species because of their sensitivity, selectivity, low cost, possibility of mass production with high reproducibility, possibility of using them as single use sensors, which avoids contamination especially in the analysis of biologic samples [4].

Electroanalytical techniques are based on electrode-solution interface monitoring any change in the ionic balance, any electron transfer in relation with the concentration of the analyte. There are three electroanalytical methods: potentiometric methods, which measure a

difference of potential at the interface of the sensor with the solution to be analyzed at zero current; voltammetric (voltamperometric) methods, which monitor the electrons transfer produced in an electrode undergoing a constant potential or which can vary in a predetermined manner; conductometric methods which observe the conductivity variation of a solution as a result of a chemical reaction.

The electrochemical investigation method used with screen printed electrodes modified with calixarenes was the cyclic voltammetry one. In this case the potential of the working electrode is scanned from an initial potential to an intermediary potential where the scanning direction of the potential is redirected towards the initial potential. The printed electrochemical cell is miniaturized and is made of three electrodes: the working electrode, the reference electrode and the auxiliary electrode. The electrodes and their contacts are put on a polyester support (125 μ m thick). The type of electroconductive inks used for their printing differs according to the type of sensor [4].

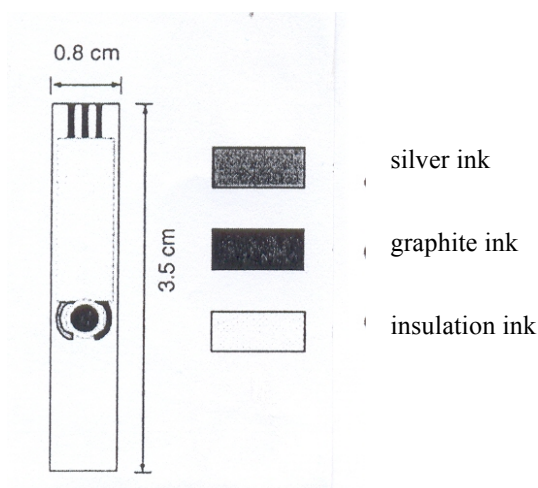


Figure 1

Screen printed electrode. The electrochemical cell is made of: working electrode (middle); counter-electrode (right); reference electrode (left) [4].

During the last years calixarenes have aroused interest among those who work in electrochemistry because of their cavitory structure, which allows them to form complex inclusions with various chemical species. The selectivity of calixarenes depends on the nature of the polar or unpolar functional groups on the edge of the cavity but also on the size of the cavity. The direct result of this potential of calixarenes was using them for creating

electrochemical sensors. The selective recognition of some cations is undoubtedly the most developed use of calixarenes. There are commercial selective electrodes which determine the sodium level in blood but the calixarenes also form complexes with potassium, cadmium, lanthanide, actinide, silver, lead, calcium, copper, zinc, mercury, being used for detecting the level of air or water pollution, in toxicology or clinical analysis [2,3,13]. Recently, sensors were constructed using calixarene also for determining some molecules. It is the case of determining folic acid from different pharmaceutical preparations containing vitamins, from human serum or from various vegetal products (asparagus, spinach, oranges), by using a carbon paste modified electrode with p-tert-butyl-calix[6]arene [12]. By using a PVC membrane sensor, modified with 4-tert-butyl-calix[n]arene ($n = 4, 6, 8$) pyridoxine hydrochloride was determined from various pharmaceutical preparations (vitamin B6) [11].

Starting from the fact that beta-blockers interact selectively with their receptors, connecting to them and blocking them, this study intends to create a connection between several beta-blockers and an artificial receptor represented by calixarene. In order to monitor the interaction between beta-blocker and calixarene, the latter was fixed on the surface of an electrode which underwent some studies of cyclic voltamperometry. All these undertakings will eventually lead to obtaining an electrochemical sensor specifically for beta-blockers.

Materials and methods

Materials

All the voltamperometric experimental measurements within this study were performed using AUTOLAB PGSTAT 30 (Ecochemie, The Netherlands) with a GPES software.

For the determination of the pH the ChemCadet pH meter was used.

All the measurements were performed at room temperature 23-25°C.

The electrodes used in the studies regarding the influence of pH upon oxidation and reduction of beta-blockers were solid electrodes purchased from BAS Inc, (West Lafayette, USA). The reference electrode was the saturated electrode Ag/AgCl (3M KCl), while the auxiliary electrode was a platinum electrode. Several electrode materials were used as working electrodes: vitreous carbon ($d=3\text{mm}$), gold and platinum ($d=1\text{mm}$).

For the studies regarding the interaction between beta-blockers and calixarene we used screen-printed electrodes C223AT type purchased from DropSens (Spain). In this case the electrochemical cell is composed of a

gold working electrode, a gold counter-electrode and a silver reference electrode. The substrate is ceramics (33 mm length, 10 mm width and 0.5 mm thickness) and the electrical contacts are made of silver. These electrodes are ideal for studies using microvolumes of solution [15].

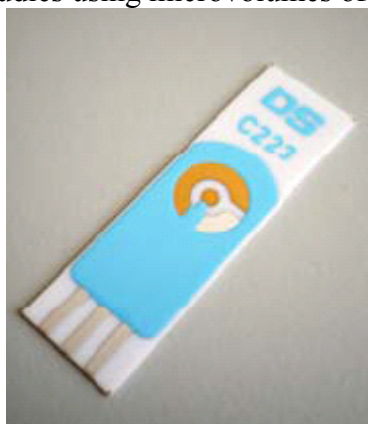


Figure 2
Screen printed electrodes, DropSens (Spain)

The six calixarenes used for this study were synthesized and structurally characterized [5,8-10]. They are presented in the table below:

Table I
Calixarenes used in this study

No.	Name	Chemical formula
1	C4Es2 p-tertbutyl-diester-calix[4]arene	
2	C4Es4 p-tertbutyl-tetraester-calix[4]arene	

Table I (continued)
Calixarenes used in this study

No.	Name	Chemical formula
3	C6Es2 p-tertbutyl-diester-calix[6]arene	
4	C6Es3 p-tertbutyl-triester-calix[6]arene	
5	C6Es4 p-tertbutyl-tetraester-calix[6]arene	
6	C6Es6 p-tertbutyl-hexaester-calix[6]arene	

$-\text{C}_4\text{H}_7\text{O}_2 = -\text{CH}_2-\text{COOC}_2\text{H}_5$

Calixarenes are soluble in chloroform; therefore 0.01M chloroform solutions were used.

The beta-blockers which were tested were atenolol, metoprolol succinate and propranolol hydrochloride from Merck and they were used as aqueous or alcoholic solutions of 0.001M or 0.01M. Metoprolol succinate and propranolol hydrochloride were dissolved in ultrapure water obtained by a Barnstead EASYpure RoDi reversed-osmosis system while the atenolol was dissolved in ethanol 20°, the pure ethanol purchased from Merck and being used with no subsequent purification.

The other substances used for preparing the phosphate buffer (diacid sodium phosphate and sodium hydroxide) were purchased from Sigma-Aldrich and were of analytical purity. The phosphate buffer was obtained from a NaH_2PO_4 0.1 M solutions to which was gradually added a NaOH 0.1M solution, constantly monitoring the pH.

Obtaining the working electrodes modified with calixarenes

The printed gold electrodes (Dropsens, Spain) were modified using the 0.01M solutions from each calixarene. By using an Eppendorf micropipette, 0.5 μL of calixarene solution were dropped twice on the surface of each gold working electrode. The solvent was left to evaporate at room temperature for 15 minutes between the two cycles as well as after.

Methods

The first part of the cyclic voltamperometric studies were performed using classical platinum, gold and vitreous carbon electrodes in aqueous medium (phosphate buffer at different pH values for metoprolol succinate and for propranolol hydrochloride) and in hydro-alcoholic medium (20% ethylic alcohol: 80% phosphate buffer for atenolol). In this case we observed the electrochemical behavior (redox) of the beta-blockers at different pH values. The reference electrode was the Ag/AgCl electrode and the auxiliary electrode was the platinum one. The current scanning for each beta-blocker and each type of electrode was performed on one hand from -1.5V passing through 0 and then going back to -1.5V and on the other hand starting from 0.6 V, passing through 0 and then going back to 0.6 V. The scan rate was 100mV/s. For metoprolol, the pH variation was from 5.22 to 8.21 and for propranolol from 4.28 to 8.10. For the atenolol hydro-alcoholic solution the pH variation was between 6.24 and 8.59. The voltamperometric cell was initially containing 10mL beta-blocker 0.01M solution to which different volumes of NaOH 0.1M were gradually added using an Eppendorf micropipette; the pH was measured under continuous stirring and afterwards the voltammograms were recorded.

The second part of the studies was performed using gold electrodes modified with calixarenes. The work was performed by dropping 25 μL of aqueous or hydro-alcoholic 0.001M beta-blockers solution on the surface of the electrode, without any added electrolyte. The conditions for the recording of cyclic voltamograms were the same as for the pH variation. Initially the recordings were made using the unmodified gold electrode for each beta-blocker and then we passed on to the electrodes modified with each calixarene and for each beta-blocker.

Results and discussion

The redox behavior of beta-blockers under the influence of pH

The redox behavior of atenolol at different pH values showed that the best recordings were performed at pH=8.59. The emerging of a reduction peak is observed at -0.334 V *versus* Ag/AgCl on the gold electrode (Figure 3a). When using the vitreous carbon electrode the results did not bring any additional information while in the case of the platinum electrode it was noticed that a more accentuated electrocatalytic effect appeared (because of the electrocatalytic properties of Pt) the oxidation and reduction processes being facilitated (it was noticed a slight shifting of the reduction and oxidation potential values towards more positive values) (Figure 3b).

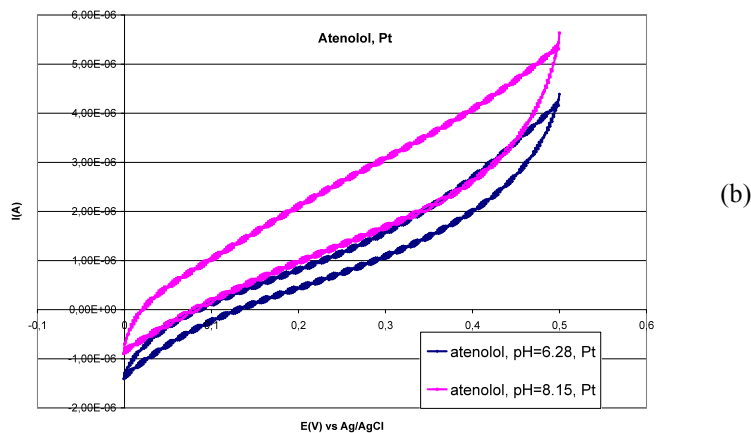
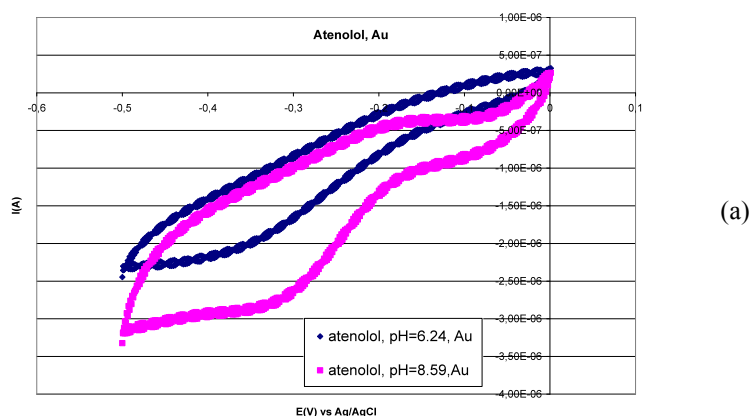


Figure 3

Electrochemical behavior of atenolol on the gold electrode (a, ♦ pH 6,24, ■ pH 8,59) and on the Pt (b, ♦ pH 6,28, ■ pH 8,15); ($v = 100$ mV/s)

Metoprolol behaved differently to acid or basic pH on the gold electrode. So, between the pH values 5.22 – 6.84 an irreversible oxidation process took place with a peak at +0.255V vs Ag/AgCl. As the pH level increased, the process tended to become reversible, with an oxidation peak at +0.255V and a reduction peak at 0.118V vs Ag/AgCl. (fig.4a). On the platinum electrode metoprolol showed notable voltammograms at pH=6.06 with an oxidation peak at -0.564V vs Ag/AgCl, while the increase of the pH, determined the decrease of the peak value at -0.681V vs Ag/AgCl. (fig.4b).

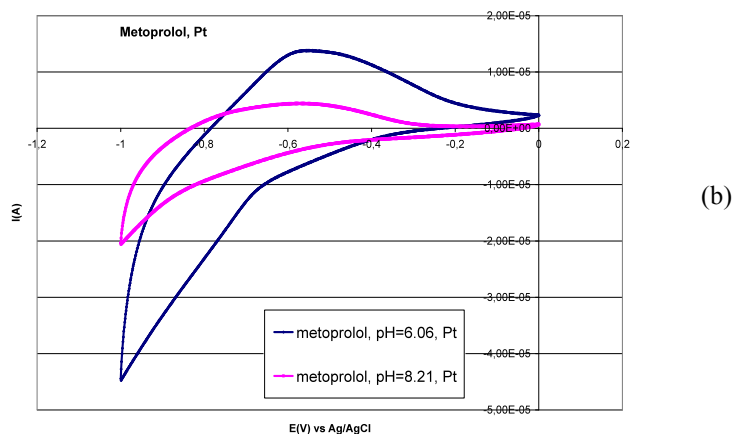
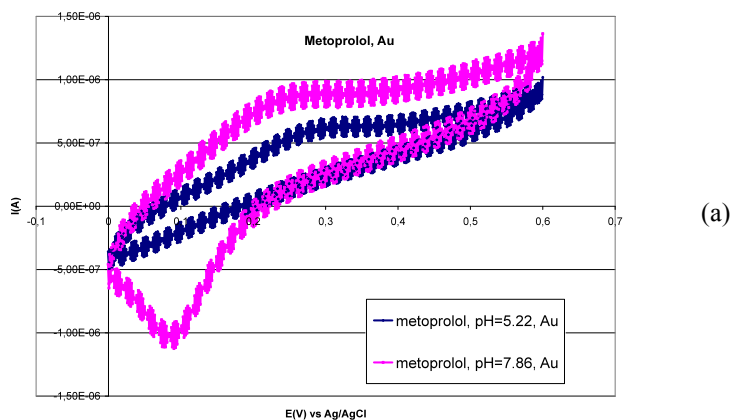


Figure 4

The electrochemical behavior of metoprolol on the gold electrode (a, ♦ pH 5,22 ■ pH 7,86) and on the platinum one (b, ♦ pH 6,06, ■ pH 8,21); $v = 100$ mV/s at different pH values

The electrochemical behavior of propranolol was only slightly influenced by the pH. On the gold electrode the signal was unmodified when changing the pH, bringing no additional information. On the vitreous carbon electrode the influence of the pH was small but there was a reduction process with a peak at -0.786V vs Ag/AgCl. On the platinum electrode an oxidation process with a peak at -0.611V vs Ag/AgCl was noticed, but as the pH increases the oxidation curve decreases.

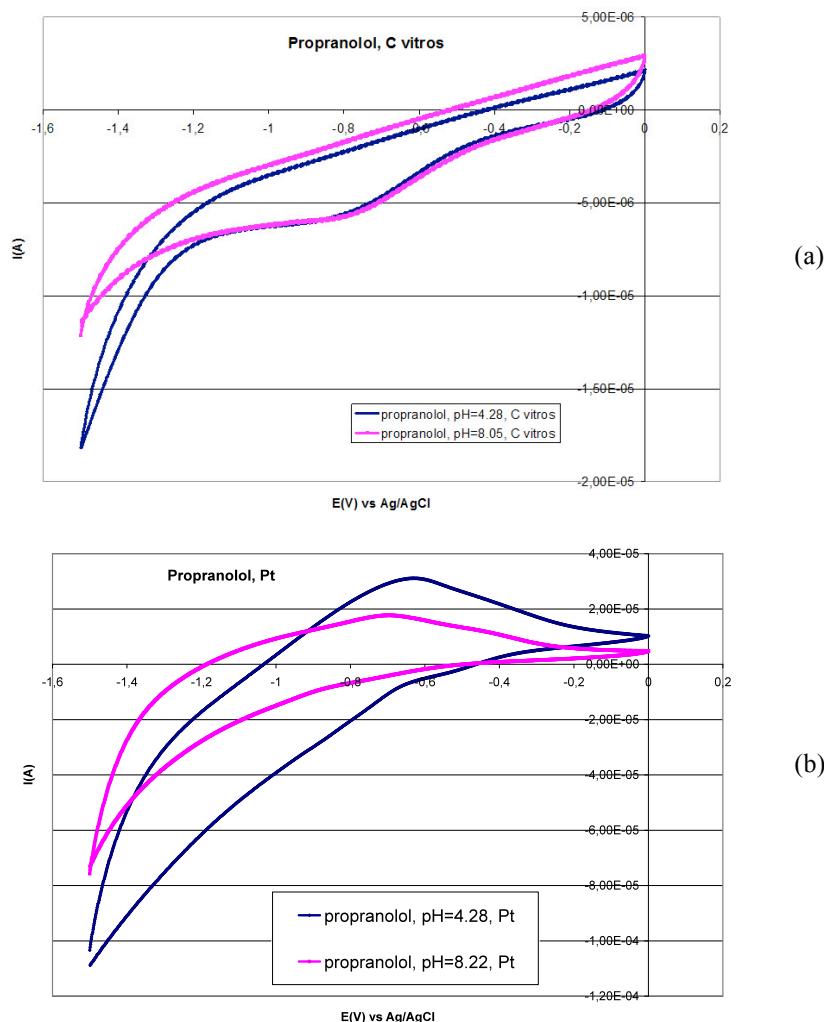


Figure 5

The electrochemical behavior of propranolol on vitreous carbon electrode (a, ♦ pH 4,28, ■ pH 8,05) and on the platinum one (b, ♦ pH 4,28, ■ pH 8,22; $v = 100\text{ mV/s}$) at different pH values

Studies made on printed gold electrodes modified with calixarene

Given the fact that the best voltammograms were recorded on the gold electrode we further studied the electrochemical behavior of beta-blockers on unmodified and modified with calixarene gold electrodes.

The voltammograms for atenolol were not very eloquent on gold electrodes. However, it was observed an oxidation peak at -0.131V vs Ag/AgCl at the unmodified electrode. If the electrode's surface is modified with calixarene C6Es2 respectively C6Es6 the signal decreases.

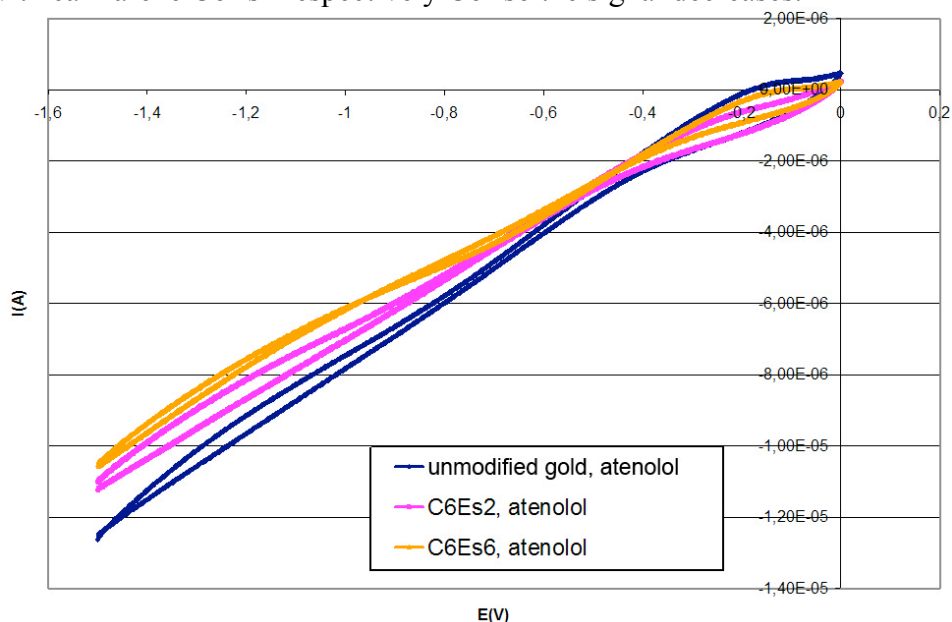
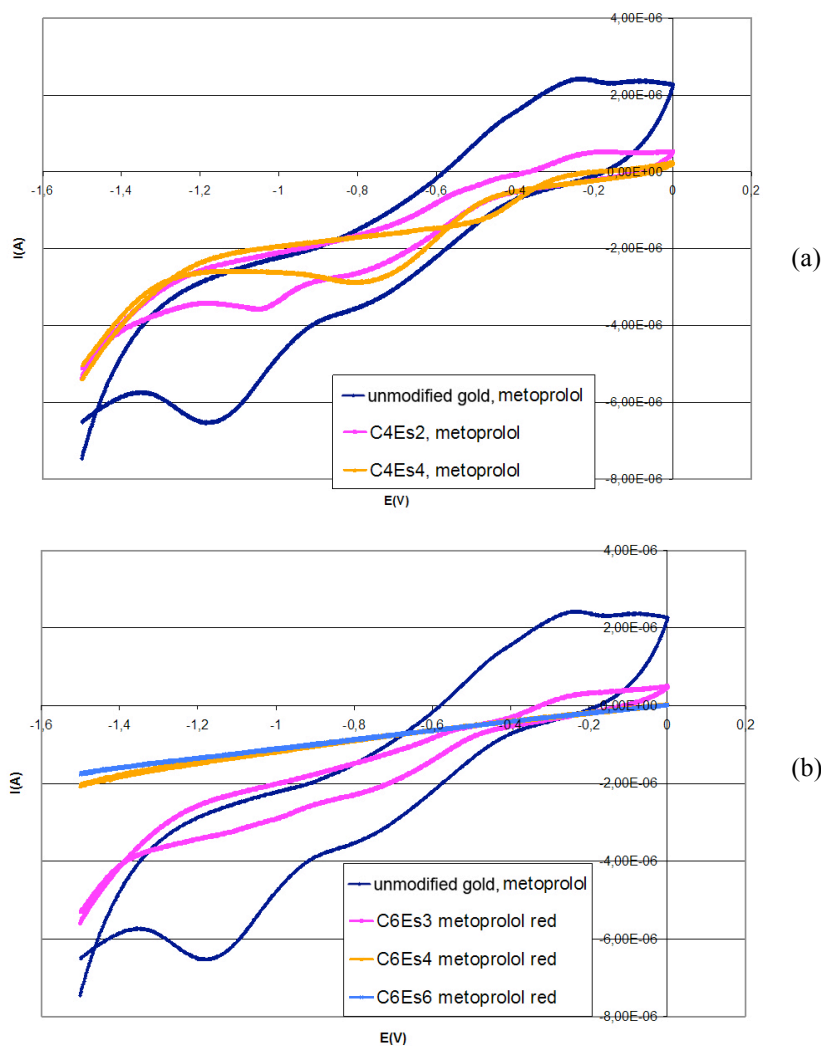


Figure 6

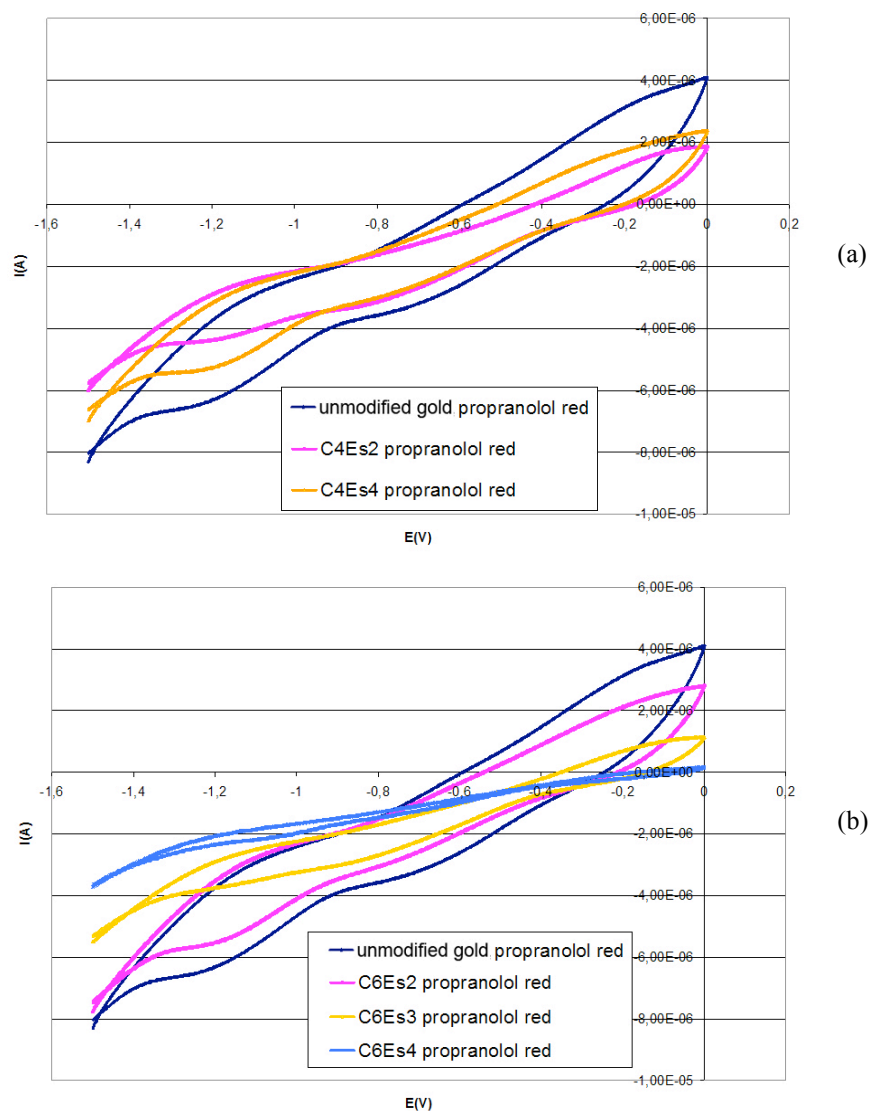
The behavior of atenolol on unmodified electrodes or electrodes modified with calixarene

On the unmodified gold electrode metoprolol had a semireversible behavior having an oxidation peak at -0.235V vs Ag/AgCl and a reduction peak at -1.180V vs Ag/AgCl. If the surface of the electrode was modified with calixarene type C4 calix[4]arene, then it was noticed that the reduction peaks shifted towards the right, at values of -1.056 V versus Ag/AgCl respectively -0.760V vs Ag/AgCl and the current's intensity decreased (fig. 7a). If the surface of the electrode was modified with calixarene type C6, calix[6]arene, then it was observed that as the number of ester functional groups increases in the calixarene structure, the intensity of the current and the redox processes decreased (fig.7b).

**Figure 7**

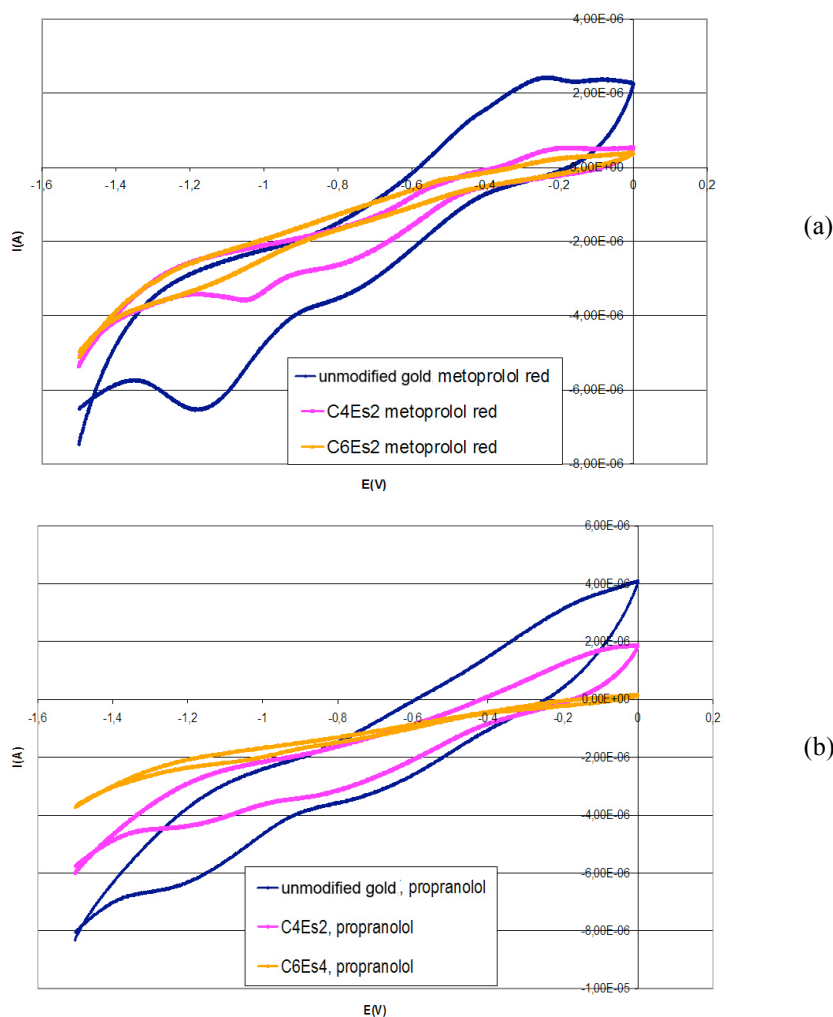
The behavior of metoprolol on the printed gold electrodes

In the case of propranolol it was also observed an oxidation peak (-0.147 V vs Ag/AgCl) and a reduction one (-1.22 V vs Ag/AgCl), when working with an unmodified electrode. When the surface of the gold electrode was modified either with calixarene type C4 or with type C6, the intensity of the current decreased as the number of ester groups in calixarene increased (figure 8).

**Figure 8**

The behavior of propranolol on printed gold electrodes

By analyzing the voltammograms obtained for metoprolol as well as for propranolol, we observed that the intensity of the signal decreased as the number of ester groups in calixarene increased but also as the number of the aromatic groups increased (figure 9).

**Figure 9**

The behavior of metoprolol (a) and propranolol (b)
on the gold electrode modified with C6 and C4.

The electrochemical behavior observed on the studied beta-blockers can be explained as follows: on one hand the increase of the host calixarene's cavity leads to the facilitation of interior insertion of the beta-blocker molecule, in this way the reduction and oxidation processes occur with more difficulty or are completely blocked, which leads to a decrease of the current's intensity. On the other hand, the increase in the number of esters in the calixarene molecule leads to the increase of the possibility of forming hydrogen links between the functional groups of calixarene and the

beta-blocker ones, so that the guest molecule is fixed inside the cavity being unable to get oxidized or reduced.

Computational Chemistry

The electrochemical behavior of beta-blockers on planar printed electrodes modified with calixarene was corroborated with the computational studies which simulate the most probable interactions, from the energetic point of view, between the calixarene derivatives (host molecule) and the studied beta-blockers (guest molecule). The spatial conformations of the molecules were obtained using the molecular mechanics method (Universal Force Field), *in vacuo*, these calculations being followed by docking studies in order to identify thermodynamically favorable 1:1 host-guest interactions (figure 10), using ArgusLab 4.0.1 software (Mark Thompson and Planaria Software LLC).

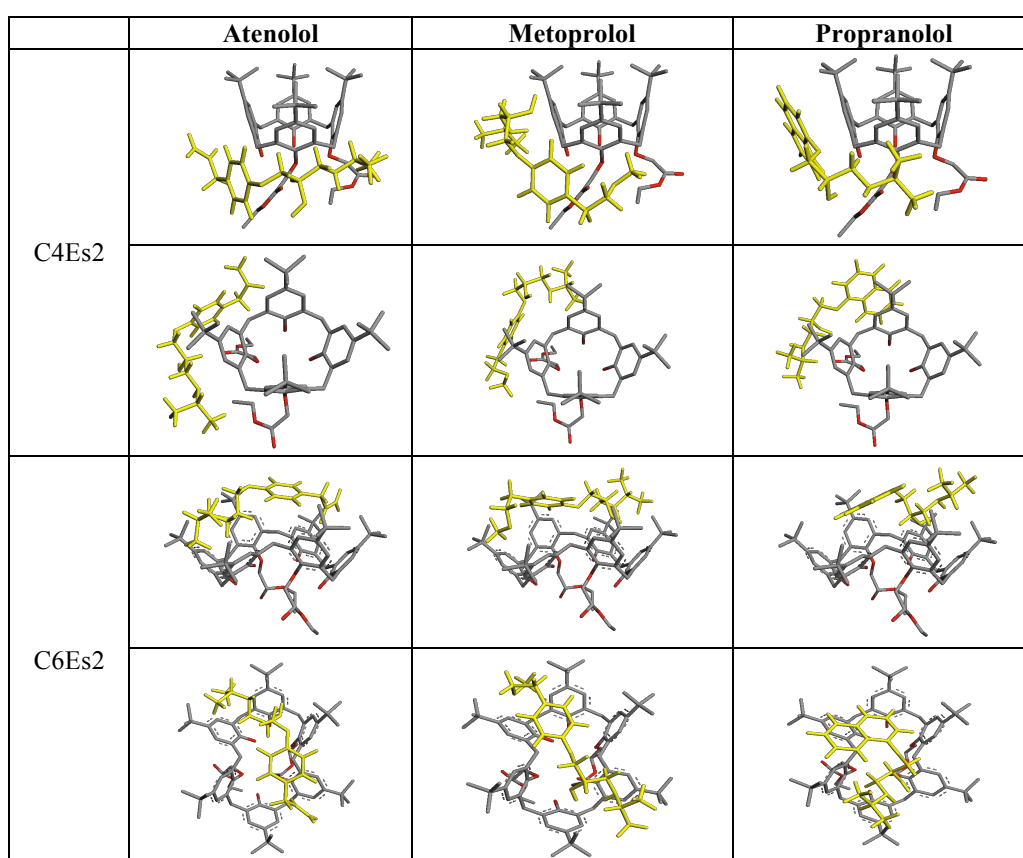
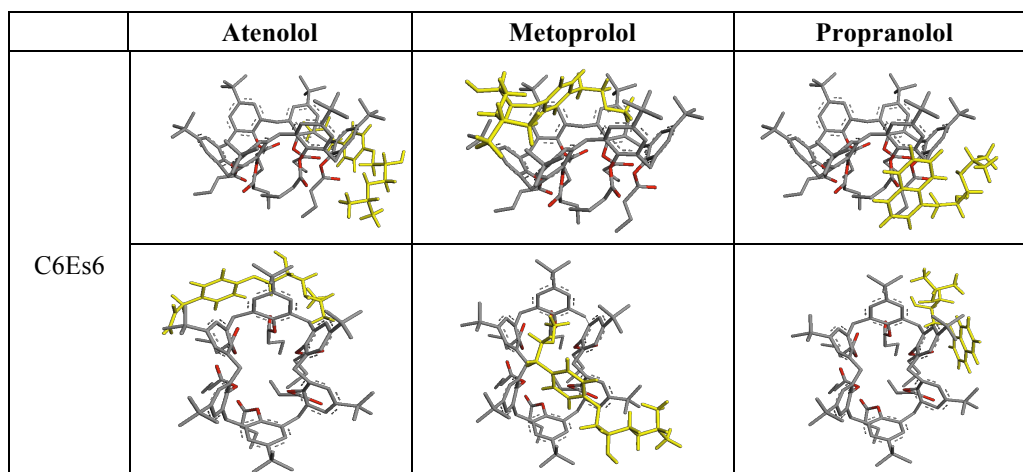


Figure 10

The steric conformations of the 1 to 1 complexes between the calixarene and beta-blocker derivatives, obtained through computational calculations *in vacuo* (ArgusLab 4.0.1, Mark Thompson and Planaria Software LLC)

**Figure 10** (*continued*)

The steric conformations of the 1 to 1 complexes between the calixarene and beta-blocker derivatives, obtained through computational calculations *in vacuo* (ArgusLab 4.0.1, Mark Thompson and Planaria Software LLC)

The results of these calculations revealed major differences in the steric interactions between beta-blockers and the studied calixarenes (1:1 complexes). Therefore, calix[4]arene derivatives, the cycle of which feature a more reduced diameter, interact with the guest molecules through the esteric moiety from outside of the cycle (exo-cavitary interaction), with small differences depending on the type of the studied beta-blocker.

In the case of calix[6]arene derivatives, depending on the molecular structure of the studied beta-blockers, the energetically favored interactions may differ. Consequently, atenolol and propranolol interact at the level of the esteric groups of the calixarene's cycle (exo-cavitary interaction), while metoprolol prefers an interaction at the hydrophobic cavity formed by the aryl-tertbutyl units (endo-cavitary interaction).

In order to establish more exactly the structure of the complex formed between the calixarene and beta-blockers derivatives we intend to perform more elaborated *ab initio* computational calculations in order to study the effect of the solvent as well as the possibility of stabilizing the clatrates through the incapsulation of one guest molecule by two host molecules (1:2 complexes). Subsequently, these results will be corroborated with those obtained by ^1H -RMN.

Conclusions

The redox properties of atenolol, metoprolol succinate and propranolol chlorhydrate were studied in the first part of the paper, also focusing on the influence of the pH. Using gold planar screen-printed electrodes modified with calixarenes it was demonstrated that there is an interaction between the host

macromolecule and the beta-blocker, so the calixarene can have the role of an artificial receptor for metoprolol, atenolol, or propranolol.

As a future direction, we consider elaborating ion-selective electrodes modified by calixarene derivatives in order to qualitatively and quantitatively determine these beta-blockers from different pharmaceutical forms or human serum.

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