

THE ELECTROCHEMICAL BEHAVIOR OF SOME LOCAL ANAESTHETICS ON SCREEN PRINTED ELECTRODES MODIFIED WITH CALIXARENES

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Abstract

The electrochemical behavior of procaine hydrochloride and benzocaine on different electrode materials, including screen-printed electrodes was investigated.

The cyclic voltamperometric studies were performed using classical platinum, gold and vitreous carbon electrodes, aiming the electrochemical behavior of local anaesthetics at different pH values (5.22-8.59). The results obtained with gold electrodes were satisfactory at neutral pH or slightly basic for benzocaine, but for procaine, an acid pH is necessary.

The second part of the studies was performed using gold screen printed electrodes modified with calixarenes. Six calixarenes derivatives of p-tert-butyl calix[4]arenes and p-tert butyl calix[6]arenes were used. In the case of using modified electrodes with calixarenes compared with the unmodified ones, it was observed that the intensity of current has decreased, due to an interaction between the calixarenes and local anaesthetics.

Rezumat

Comportamentul electrochimic al clorhidratului de procaină și al benzocainei a fost investigat pe diferite materiale electrodice, inclusiv electrozi imprimați serigrafic.

Studiile de voltamperometrie ciclică au utilizat electrozi clasici de platină, aur și carbon vitros, urmărindu-se comportamentul electrochimic al anestezicelor locale la diferite valori de pH (5,22-8,59). Rezultatele obținute au fost satisfăcătoare, dacă se folosește electrodul de aur la pH neutru sau slab bazic pentru benzocaină. Pentru procaină este necesar un pH acid.

A doua parte a studiilor a utilizat electrozi de aur planari imprimați modificați cu calixarene. S-au utilizat șase derivați de p-terț-butil calix[4]arena și p-terț-butil-calix[6]arenă. În cazul utilizării electrozilor modificați cu calixarene față de cei nemodificați se observă că intensitatea curentului scade, datorită interacțiunii dintre calixarene și anestezicele locale.

Keywords: calixarenes, local anaesthetics, screen printed electrodes, cyclic voltammetry

Introduction

Local anaesthetics are a group of compounds that, at proper concentrations in the nervous tissue, reversibly block the influx conduction. The local anaesthetics used in this study were benzocaine and procaine hydrochloride.

Benzocaine (figure 1) is a local anaesthetic used for reducing the pain in the mucous membranes and teguments. Numerous pharmaceutical products contain benzocaine: sucking tablets, syrups, solutions used for the treatment of oral or tongue ulcerations, gastric ulcer, as well as ointments and suppositories used for treating hemorrhoidal disorders [2].

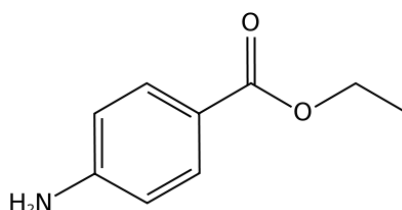
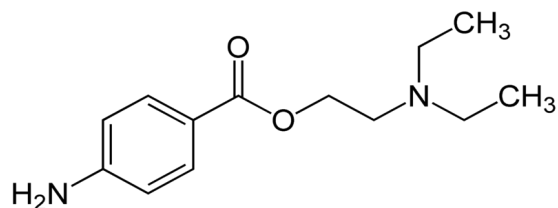


Figure 1

The chemical structure of benzocaine

A considerable number of studies describe the different strategies used for quantifying benzocaine in tissues, biological fluids, as well as in different pharmaceutical forms. Most of them are based on high performance liquid chromatography, which can be combined with gas-chromatography or mass spectroscopy [4]. More recent studies can determine local anaesthetics in the body fluids using gas-chromatography combined with surface ionization detection [5]. Other research studies led to the identification and quantification of procaine, benzocaine and tetracaine using sequential injection analysis combined with chemiluminescence detection [6].

Procaine (Figure 2) is an injectable local anaesthetic with short-term effect, used for reducing the pain (in neuralgia, myalgia and arthralgia) before surgery or postoperative. Procaine also has a systemic effect: analgesic, vasodilator, weak antispasmodic and general trophic [2].

**Figure 2**

The chemical structure of procaine

There is a lot of information in the domain literature about the detection of procaine approaching colorimetric, spectrophotometric, chromatographic and electrochemical methods [5, 10]. The American Pharmacopoeia recommends the spectrophotometric method with a maximum absorption at 280 nm. Liquid chromatography combined with mass spectroscopy as well as HPLC are selective, accurate methods, with a low detection limit, but they are expensive and require a long analysis time [10].

Recent studies approached the detection of procaine using the advantages of electrochemistry: high degree of selectivity, rapid response and simplicity. Kataký and Palmer [3] produced an ion-selective electrode for procaine, which has a detection limit at 10^{-5} mol/L. Wang and collaborators obtained a detection limit at 5×10^{-8} mol/L using differential pulse voltammetry and a modified carbon paste electrode. Wu and collaborators [8] used an electrode covered with carbon nanotube films, with a 4 minute adsorption before applying the stripping voltammetry. Bergamini and collaborators [9] studied the electrooxidation of procaine using a carbon based screen-printed electrode (SPE) and performed the amperometric determination of procaine using the flow injection method. The method was optimized by evaluating the parameters' influence (the applied potential, pH, scan rate, analyte volume) and it was afterwards applied in determining procaine in the pharmaceutical forms.

Due to their various advantages, printed electrodes (SPE) were increasingly used during the last decade in biomedical and pharmaceutical analysis, in the analysis of environmental probes and foods, progressively replacing the use of mercury electrode or other classic solid electrodes. These electrochemical sensors became increasingly used for detecting different ionic and/or molecular species due to their sensitivity, selectivity, low cost, possibility of mass production with high reproducibility, possibility of using them as single usage sensors, which avoids contamination especially in the analysis of biologic samples.

The electrochemical investigation method for observing the behavior of benzocaine and procaine, using printed electrodes modified with calixarene was the cyclic voltammetry method. 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. [11].

Calixarenes are macrocycles with a cavity structure which allows them to form complex inclusions with numerous chemical species. The selectivity of calixarene depends on the nature of the polar or non-polar functional groups which are grafted on the edge of the cavity but also on the size of the cavity of the macromolecule. Given these remarkable qualities, calixarene are increasingly used in electrochemistry for creating electrochemical sensors.

It is commonly known that calixarene derivatives are used for the electrochemical determination of some cations, from alkaline metals to lanthanides and actinides. These sensors have applications in the detection of the level of air or water pollution, in toxicology or clinical analysis [7].

Recently, sensors were constructed using calixarenes also for determining some organic molecular compounds. 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 [17]. By using a PVC membrane sensor, modified with 4-tert-butyl-calix[n]arene (n=4, 6, 8) pyridoxine chlorohydrate was determined from various pharmaceutical preparations (vitamin B6) [16].

Selectivity was obtained by using a potentiometric sensor with calix[6]arene (carboxymethyl derivative) for non-ramified primary amine (2-phenyl-ethyl-amine), in their protonated form. The explanation of the selectivity was based on the links between the -NH_3^+ group of the "guest" and the oxygen atoms from the carbonyl group of the "host." The selective linking of dopamine compared to adrenaline and noradrenalin is very interesting from the bioanalytical point of view, but the interference with the potassium ions led to the search of other calixarenes which are more selective. Homooxacalix[3]arene was recently discovered to be very selective for dopamine and does not interfere with other neurotransmitters, with potassium or sodium [1].

The study of the electrochemical behavior of benzocaine and procaine using screen-printed electrodes modified with calixarene will allow the highlighting and monitoring of the potential interactions between them and “artificial” receptors. This will also bring new information regarding the mechanisms of action of local anaesthetics while offering a new method of screening the pharmacotherapeutic action of the new natural or synthesized medicinal substances.

Materials and Methods

Materials

All the voltamperometric experimental measurements were made at room temperature, 23-25 °C, using AUTOLAB PGSTAT 30 (Ecochemie, The Netherlands) equipped with GPES software.

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

The electrodes used in the studies regarding the influence of pH upon oxidation and reduction of anaesthetics, 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: glassy carbon (d=3mm), gold and platinum (d=1mm).

For the studies regarding the interaction between anaesthetics and calixarene we used screen-printed electrodes C223AT type made by 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 micro-volumes of solution [18].

The six calixarenes used (Table I) were synthesized and fully characterized structurally [12, 13, 14, 15], being used in chloroform solutions of 0.01 M.

The anaesthetics which were tested were benzocaine and procaine hydrochloride from Merck. They were used as water or alcohol solution of 0.001M or 0.01 M. Procaine hydrochloride was dissolved in ultrapure water (18.2MΩ·cm) obtained by using Barnstead EASYpure RoDi device, while benzocaine was dissolved in phosphate buffer containing 20% v/v ethanol, the ethanol coming from Merck and being used with no subsequent purification.

Table I
The calixarenes used

No.	Name	Chemical structure
1	C4Es2 p-tert-butyl-diester-calix[4]arene	
2	C4Es4 p-tert-butyl-tetraester-calix[4]arene	
3	C6Es2 p-tert-butyl-diester-calix[6]arene	
4	C6Es3 p-tert-butyl-triester-calix[6]arene	
5	C6Es4 p-tert-butyl-tetraester-calix[6]arene	
6	C6Es6 p-tert-butyl-hexaester-calix[6]arene	

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

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

Calixarenes modified electrodes preparation

The printed gold electrodes were modified using the 0.01M solutions of calixarenes. By using an Eppendorf micropipette, 0.5 μL of calixarenes solution was 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 droppings 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 (procaine hydrochloride in phosphate buffer at different pHs) and in hydro-alcoholic medium (20% ethyl alcohol: 80% phosphate buffer for benzocaine). In this case we observed the electrochemical behavior of the anaesthetics at different pH values. The potential sweep for each anaesthetic 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 scanning speed was of 100mV/s. The benzocaine hydro-alcoholic solution had the pH between 4.86 and 8.30, while the procaine hydrochloride solution had the pH between 4.38 and 8.10. The voltamperometric cell was initially filled with 10ml anaesthetic solution 0.01M to which different volumes of NaOH 0.1M were gradually added using an Eppendorf micropipette; the pH was measured under agitation and afterwards the voltammograms were recorded.

The second part of the studies was performed using screen printed gold electrodes modified with calixarene. The assays were performed in a drop of solution, adding 25 μL of aqueous or hydro-alcoholic 0.001M anaesthetic solution on the surface of the electrode, without containing an electrolyte. The conditions for the recording of cyclic voltammograms were the same as for the pH variation. Initially the recordings were made using the unmodified gold electrode for each anaesthetic and then we passed on to the electrodes modified with each calixarene and for each anaesthetic. On the gold printed electrodes we also observed the electrochemical behavior of procaine at different scan rates. The working conditions were kept the same: the assays on the unmodified gold electrodes were performed in a drop of

solution, changing only the scan speed as follows: 0.020 V/s, 0.050 V/s, 0.075 V/s, 0.100 V/s, 0.150 V/s and 0.200 V/s.

Results and Discussion

The redox behavior of anaesthetics under the influence of pH

The behavior of benzocaine varied according to the nature of the electrode material as well as of the pH. On the vitreous carbon electrode the influence of pH was very low, and we noticed an overlapping of the obtained voltammetric curves (Figure 3a). In the case of the platinum electrode, the basic pH of 8.30 emphasized a reversible process, with an oxidation peak at -0.850V and a reduction one at -0.860V compared to the Ag/AgCl reference electrode (fig. 3b).

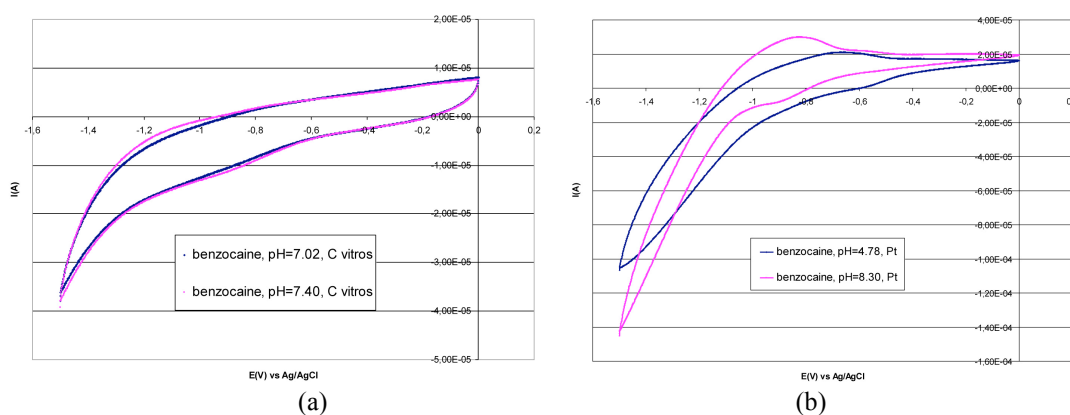
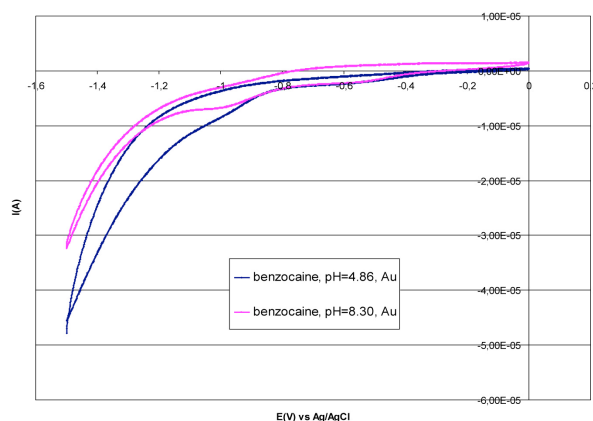


Figure 3

The electrochemical behavior of benzocaine on (a) vitreous carbon electrode, pH=7.02 respectively 7.40 and (b) platinum electrode, pH=4.78 respectively 8.30. The scan rate was 100mV/s.

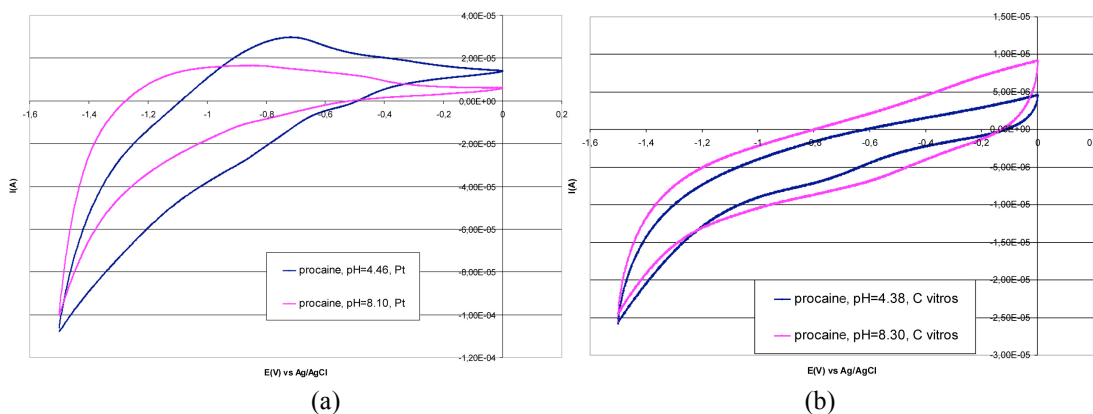
On the gold electrode, (Figure 4), the redox process of benzocaine was very weak, while as the pH increased this process was completely obstructed due to the deprotonation of the investigated molecule. We noticed a weak reduction peak at -0.997V at pH 4.86 and at -0.974V at pH 8.30 compared to the Ag/AgCl electrode.

On the platinum electrode, procaine hydrochloride showed an oxidation peak which shifts from -0.721V to -1.074V vs. Ag/AgCl as the pH increased.

**Figure 4**

The electrochemical behavior of benzocaine on the gold electrode, pH=4.86 and 8.30, respectively. Scan rate 100mV/s

On the vitreous carbon electrode, the redox processes were facilitated at the increase of pH, so that at pH 8.30 there was a reduction peak at -0.633V, compared to the Ag/AgCl electrode. (Figure 5)

**Figure 5**

The electrochemical behavior of procaine on (a) platinum electrode, pH=4.46 and 8.10, respectively and (b) vitreous carbon, pH=4.38 respectively 8.30. Scan rate 100mV/s

The electrochemical behavior of procaine on the gold electrode (Figure 6) was reversed compared to the vitreous carbon electrode; meaning that the intensity of the reduction and oxidation peaks, decreased when the pH increased. An optimum voltammogram was recorded at pH 4.33 with a reduction peak at 0.493 V vs. Ag/AgCl.

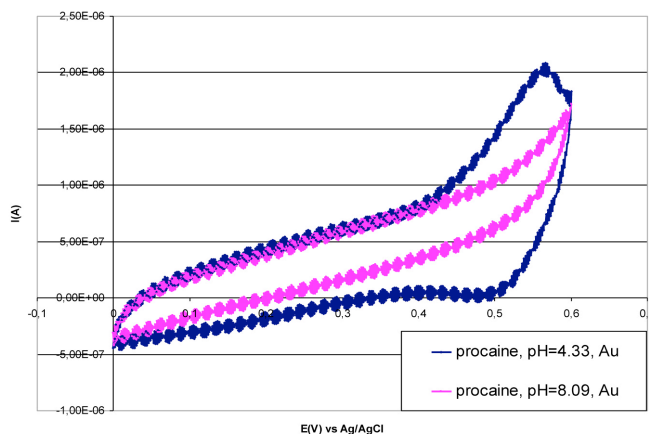


Figure 6

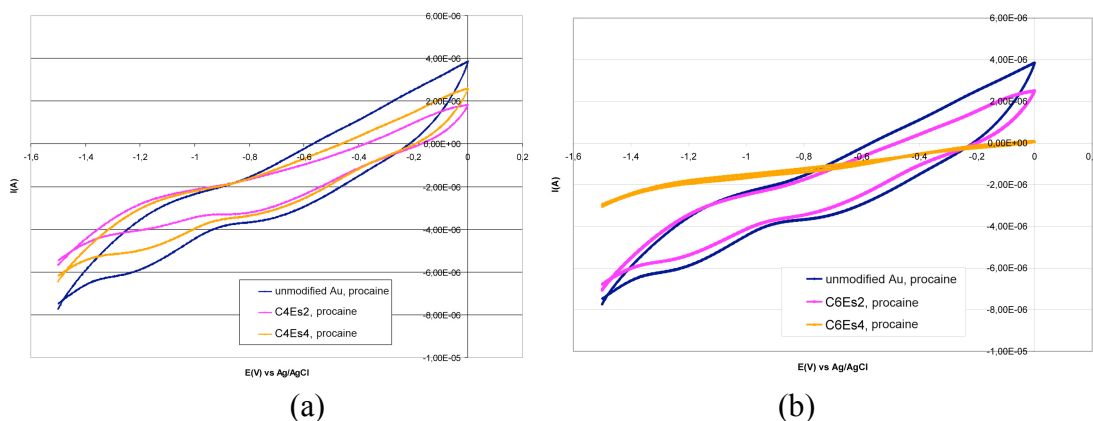
The electrochemical behavior of procaine on the gold electrode pH=4.33 and 8.09, respectively; scan rate 100mV/s

We observed the electrochemical behavior of the two anaesthetics related to two variable parameters: pH and type of electrode. Consequently we can affirm that the most interesting electrochemical behavior of benzocaine was in the range of neutral towards alkaline pH. As for procaine, it needs an acidic pH. Regarding the type of electrode, the best electrochemical responses for procaine were recorded on the gold electrode. On the other hand, benzocaine was more difficultly oxidized and reduced on those electrodes, favoring the platinum electrode.

Electrochemical study on gold screen printed electrodes modified with calixarenes

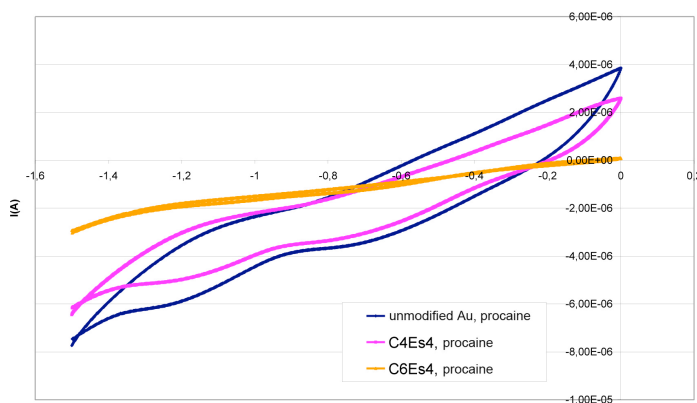
As we noticed in the study of pH, when working with the solid gold electrode, benzocaine was oxidized and reduced with great difficulty. Therefore, the information brought by benzocaine on the screen printed gold electrode was unsatisfactory. There was no observable electrochemical signal both on the unmodified electrode as well as on the electrode modified with calixarenes.

However, procaine hydrochloride showed an oxidation at -0.984 V vs. Ag/AgCl and a reduction at -1.243V vs. Ag/AgCl. By modifying the electrode with C4 calixarene the redox signal decreased (Figure 7a). If the modification was done with C6 calixarene we observed the same decrease of the current intensity as the number of grafted ester groups of calixarene increased (Figure 7b).

**Figure 7**

The behavior of procaine on gold printed electrodes modified with (a) C4Es2 respectively C4Es4 and (b) C6Es2 respectively C6Es4. Scan rate 100mV/s.

If we relate to the same number of ester groups grafted on the calixarenes structure and we only modify the number of aromatic groups of calixarenes, then procaine will link more strongly to the calixarenes having six aromatic groups rather than those with four groups. Voltammograms showed a decrease in the intensity of the current for the electrodes modified with C4 calixarene, and this decrease was more accentuated for the electrodes modified with calixarene C6 (Figure 8).

**Figure 8**

The behavior of procaine on printed electrodes modified with calixarene C4 and C6. Scan rate 100mV/s.

In conclusion, the interaction of procaine hydrochloride with calixarenes on the gold screen printed electrodes led to a decrease in the intensity of the current with the increase of the number of grafted ester

groups and the of calixarene's number of aromatic cycles. This can be explained by the fact that the host (calixarene), having a larger cavity and a higher possibility of bonding with the guest molecule, prevents the reduction and oxidation of procaine at the surface of the electrode, which leads to the decrease of the signal.

The influence of scan rate variation upon the electrochemical behavior of procaine on the gold printed electrodes

Because of the fact that on gold printed electrodes benzocaine did not perform a remarkable redox behavior, in the study of the scan rate variation we only worked on procaine hydrochloride.

Unmodified gold planar printed electrodes were used, varying the scan rate from 0.020 V/s up to 0.200 V/s. Otherwise, the working conditions were kept the same: the work was performed in a drop of solution; the current scan was performed starting from -1.5V, passing through 0 and going back to -1.5V.

As it was experimentally observed, an increase in the scan rate only leads to an accentuation of the oxidation and reduction processes. In the case of procaine the reduction peak was recorded at -1.12 V, and the oxidation one at -0.303 V compared to the Ag/AgCl electrode, with a scan rate at 0.020 V/s. At a scan rate higher than 0.200 V/s, the intensity of the current increased.

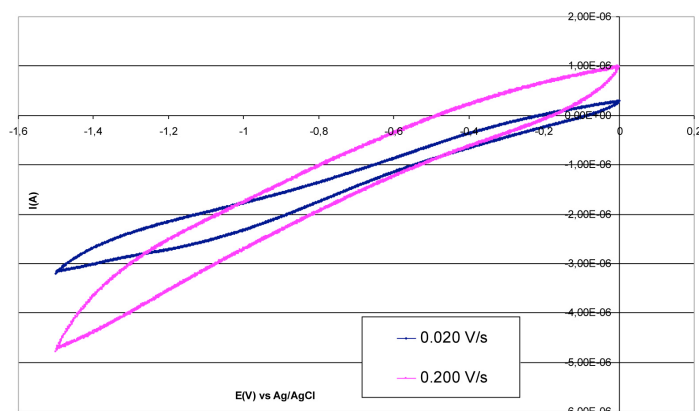


Figure 9.

The influence of scan rate variation upon the electrochemical behavior of procaine chlorohydrate. Unmodified gold printed electrode, scan rate 0.02 V/s and 0.200 V/s respectively

Conclusions

We studied the redox proprieties of benzocaine and procaine hydrochloride, also observing the influence of pH upon the electrochemical behavior.

By using gold screen printed electrodes modified with calixarene we showed the existence of an interaction between the host macromolecule and the local anaesthetics studied, therefore, calixarenes can have the role of an artificial receptor for procaine hydrochloride.

As further studies, we take into consideration creating selective ion electrodes with PVC membrane for the qualitative and quantitative determination of local anaesthetics from different pharmaceutical forms.

Acknowledgements

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