

## Bottom up approaches for amino $\beta$ -CD adsorption on gold surfaces. A comparative study



Marycarmen García<sup>a</sup>, Soledad Bollo<sup>a</sup>, Gustavo A. Rivas<sup>b</sup>, Nancy F. Ferreyra<sup>b,\*\*</sup>,  
Claudia Yáñez<sup>a,\*</sup>

<sup>a</sup> Centro de investigación de Procesos Redox, CIPRex, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile

<sup>b</sup> INFIQC, Departamento de Físicoquímica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, 5000 Córdoba, Argentina

### ARTICLE INFO

#### Article history:

Received 1 February 2016

Received in revised form 5 April 2016

Accepted 7 April 2016

Available online 8 April 2016

#### Keywords:

cyclodextrins

gold modification

diazonium salt electrochemistry

self-assembled monolayers

surface plasmon resonance

### ABSTRACT

The immobilization of three amino- $\beta$ -cyclodextrin derivatives on gold surfaces via different bottom-up approaches was evaluated using cyclic voltammetry and surface plasmon resonance. Direct adsorption on gold and covalent attachment via amide bond formation with carboxylic groups of both the self-assembled monolayer of 4-mercapto benzoic acid (4-MBA) and the electrografted diazonium salt synthesized *in situ* from 4-amino benzoic acid (4-ABA) were tested. Amino  $\beta$ -CD with 1, 2 or 7 amine groups on the primary rim were used. The influence of the immobilization methodologies on the electrochemical properties of the resulting surfaces was studied using hydroquinone and ferricyanide as redox probes. The results indicate that the surface coverage of the molecules as well as their organization at the gold substrate depends on the number of amine groups on the primary rim of  $\beta$ -CD. A higher coverage via direct adsorption is obtained using the 7-amino groups derivative, while the highest coverage is reached with the 2-amino groups derivative via covalent attachment at diazonium salt electrografted surfaces. A critical discussion about the influence of different strategies used for the immobilization and the number of terminal amine groups on the  $\beta$ -CD structure is performed.

© 2016 Elsevier Ltd. All rights reserved.

## 1. Introduction

Cyclodextrins (CDs) are organic macrocycles that possess a hydrophobic toroidal cavity and are capable of forming inclusion complexes with hydrophobic molecules of compatible sizes [1]. Due to their unique properties, CDs have been used in different applications, including drug carriers in the pharmaceutical industry [2,3], catalysis [4], analytical separations [5,6], and more recently, in the design of biomimetic systems and the construction of supramolecular architectures and molecular machines [7,8].

Several strategies have been employed for the immobilization of CDs on different surfaces, being the chemisorption of thiolated

CDs on gold surfaces the most widely known [9–18]. The incorporation of multiple and symmetrically distributed thiol groups on the CD increases the possibility of achieving a proper orientation of the molecule once anchored in a given surface, but it can result in poor packing of the monolayer. The functionalization of CDs with long alkyl thioethers as spacers allows obtaining self-assembled structures with improved surface coverage and order [19]. However, the incorporation of alkyl chains creates an insulating layer that hampers their use in applications based on electron transfer processes [20].

As an alternative, several authors have recently explored the adsorption of CD diazonium salt derivatives. In this approach, some functional groups of the CDs are substituted by aromatic amines for a *posteriori* diazonium salt generation either *ex situ* or *in situ* using adequate reducing conditions. The electroreduction of the diazonium salt at conductive or semiconductive surfaces produces a radical that forms a strong covalent bond ensuring a stable surface coating [21]. Tang et al. [22] have described the synthesized phenylamino- $\beta$ CD and its linkage to the carbon surface by electrografting of its diazonium salt generated *in situ*. Hinge

\* Corresponding author at: CIPRex, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, P.O. Box 233, Santiago, Chile.

\*\* Corresponding author at: INFIQC, Departamento de Físicoquímica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, 5000, Córdoba, Argentina. Tel.: +54 351 4334169; fax: +54 351 4334188.

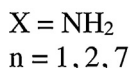
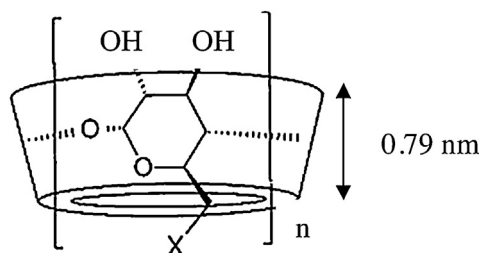
E-mail addresses: [ferreyra@fcq.unc.edu.ar](mailto:ferreyra@fcq.unc.edu.ar) (N.F. Ferreyra), [cyaneyz@ciq.uchile.cl](mailto:cyaneyz@ciq.uchile.cl) (C. Yáñez).

et al. [23] have recently proposed the synthesis and purification of  $\beta$ -CD diazonium salt derivatives to modify glassy carbon and gold surfaces by electroreduction in organic solvents. This method requires the synthesis and isolation of pre-functionalized CDs, limiting the construction of CD assemblies to the real synthetic possibilities.

An alternative way to overcome this problem is to build the platform via a bottom-up approach using surfaces modified with functional groups able to be linked to the CDs. A few examples of this strategy have been reported. So, Willner et al. [24] have described the adsorption of amino- $\beta$ -CD on gold surfaces previously modified with a monolayer of dithiobis (succinimidyl propionate) based on the procedure proposed by Katz [25]. Campiña et al. [26] have immobilized  $\beta$ -CD on gold surfaces modified with 11-amino-1-undecanethiol by activation of the exposed functional groups with di-(N-succinimidyl) carbonate. Reed et al. [27] have prepared  $\beta$ -CD monolayers on silicon nanowires silanized with 3-aminopropyltriethoxysilane and subsequently allowed to react with p-phenylenediisothiocyanate and amino-functionalized  $\beta$ -CD. Huskens et al. [20] have evaluated the effect on the kinetics of the self-assembly process, as well as the structural and electrochemical properties, of monolayers of CDs completely functionalized in their primary rim with iodide, nitrile, amine, isothiocyanate, methyl sulfide, and isocyanide groups. Apart from this latter work, the number of studies comparing different routes to immobilize CDs is rather scarce.

The aim of the present work was to evaluate the immobilization of three amino- $\beta$ -CD on gold surfaces via different bottom-up approaches, with a critical discussion about the influence of the surface used as platform for the immobilization. The effect of the number of terminal amine groups linked to the  $\beta$ -CD on the amount of molecules incorporated to the surface was analysed by surface plasmon resonance (SPR) and by studying the electrochemical behaviour of different redox markers.

The immobilization of amino  $\beta$ -CD (with 1, 2 or 7 amine groups on the primary rim, Scheme 1) on gold surfaces was carried out using different methodologies: i) direct adsorption (Scheme 2A); ii) amide-bond formation with the carboxylic groups of a self-assembled monolayer of 4-mercapto benzoic acid (4-MBA) (Scheme 2B); and iii) amide bond formation with the carboxylic groups of the electrografted diazonium salt synthesized *in situ* from 4-amino benzoic acid (4-ABA) (Scheme 2C). The resulting surfaces were characterized by cyclic voltammetry using different redox markers and surface plasmon resonance. The effect of the number of amino groups linked to the  $\beta$ -CD and the influence of the method of immobilization on the properties of the resulting surfaces are discussed in the following sections.



Scheme 1. Molecular structure of the amino  $\beta$ -cyclodextrins.

Summarizing, the strategies presented in this work try to open an innovative alternative to immobilize  $\beta$ -CD on gold electrodes without the requirement of synthesizing the thiol or diazonium-modified cyclodextrins. These important advantages of the supramolecular architectures, which we will describe here, could represent a significant contribution in the field of host-guest interactions opening the door to future analytical devices.

## 2. Materials and Methods

### 2.1. Reagents

All reagents were analytic grade; NaNO<sub>2</sub> was from Anedra, 4-amino benzoic acid (4-ABA), 4-mercapto benzoic acid (4-MBA), hydroquinone (H<sub>2</sub>Q), K<sub>3</sub>Fe(CN)<sub>6</sub>, N-hydroxysulfosuccinimide (NHS), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC), and ethanalamine chlorohydrate (EtOH-NH<sub>2</sub>) were from Sigma-Aldrich. 6-monodeoxy-6-monoamino- $\beta$ -cyclodextrin, ( $\beta$ -CD<sub>1</sub>, MW = 1134 free base), 6-dideoxy-6-diamino- $\beta$ -cyclodextrin, ( $\beta$ -CD<sub>2</sub>, MW = 1133 free base) and heptakis-(6-amino-6-deoxy)- $\beta$ -cyclodextrin ( $\beta$ -CD<sub>7</sub>, MW = 1128 free base) containing 1, 2 and 7 amine terminal groups, respectively, were from AraChem (Netherlands). In the text, amino- $\beta$ -cyclodextrin are represented as  $\beta$ -CD<sub>n</sub> (where n indicates the number of amine terminal groups of the  $\beta$ -CD derivative). Other chemicals were reagent grade and used without further purification. All solutions were prepared with ultrapure water (18 M $\Omega$  cm) from a MilliRO-MilliQ system.

### 2.2. Equipment

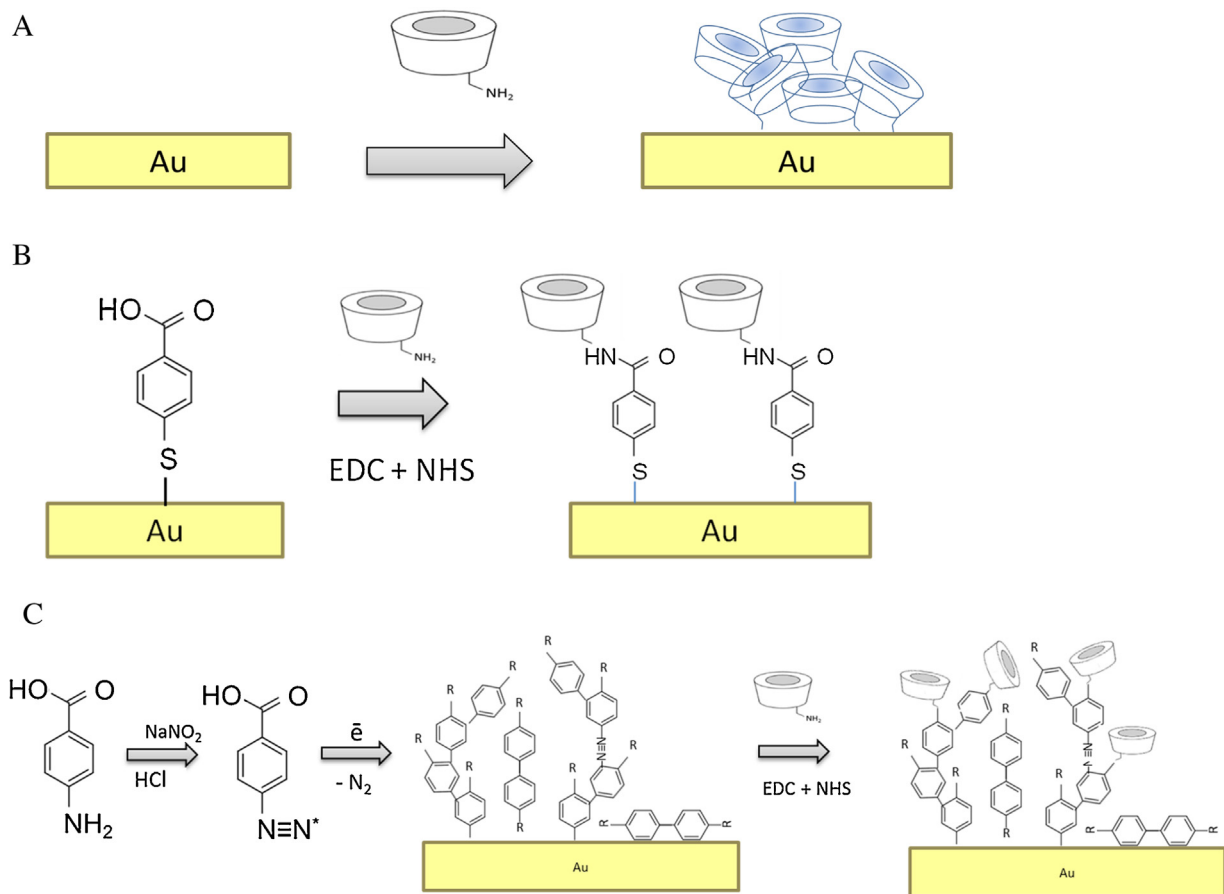
Cyclic voltammetry (CV) was performed using an Autolab PGSTAT 128 N potentiostat (Metrohm Autolab B. V.) and a CHI-Instruments 650 potentiostat. Gold disk electrodes (Au) with 2 mm diameters (Model CHI 101) were used as working electrodes. A platinum wire and a Ag/AgCl, 3 M NaCl electrode (Model RE-5B, BAS) were used as counter and reference electrodes, respectively. All reported potentials refer to this reference electrode.

Surface plasmon resonance (SPR) measurements were conducted using a single channel Autolab SPRINGLE instrument (Metrohm Autolab B. V.). Gold SPR sensor disks (Model BK 7) were mounted on a hemicylindrical lens through index-matching oil to form the base of the cuvette. The measurements were conducted under non-flow liquid conditions with the cuvette thermostated at 25 °C using a volume sample of 100  $\mu$ L.

### 2.3. Surface modification

#### 2.3.1. Gold surfaces treatment

**Gold electrodes.** The gold disk electrodes were cleaned before each experiment by successive mechanical, chemical and electrochemical treatment. The mechanical treatment consisted of polishing with 0.05  $\mu$ m alumina for 6 min, followed by sonication in deionized water for 5 min. The chemical treatment was performed by immersion in "Piranha" solution (1:3 v/v H<sub>2</sub>O<sub>2</sub>/H<sub>2</sub>SO<sub>4</sub>) for 5 min, followed by sonication for 10 seconds in an ultrasonic bath and rinsing with ultrapure water. The electrochemical treatment consisted in the application of two consecutive step potentials of 2.0 V and -1.0 V for 2 and 4 seconds, respectively, using 0.500 M H<sub>2</sub>SO<sub>4</sub> as supporting electrolyte. The state of the surface after the pretreatment was evaluated by cyclic voltammetry at 0.100 Vs<sup>-1</sup> in the 0.500 M H<sub>2</sub>SO<sub>4</sub> solutions. The real surface area and roughness factor were determined from the corresponding voltammograms by the integration of the gold oxide reduction peak [28]. The roughness factor of the electrodes used in this work was 2.3  $\pm$  0.4.



**Scheme 2.** Scheme of the surface modification methods: A) Direct adsorption of amino- $\beta\text{-CD}_n$  on the gold surface, B) Covalent attachment by amide bond formation with the carboxylic groups of a self-assembled monolayer of 4-mercapto benzoic acid and C) Covalent attachment by amide bond formation with the carboxylic groups of the electrografted diazonium salt from 4-amino benzoic acid.

**SPR sensor disks.** The SPR sensor disks were cleaned before each experiment by washing exhaustively with ethanol and pure water.

### 2.3.2. Cyclodextrins immobilization protocols

An illustration of the procedures used to immobilize the  $\beta\text{-CD}_n$  on gold surface is presented in the [Scheme 2](#).

**Scheme 2A: Adsorption at open circuit potential (ocp).** The  $\beta\text{-CD}_n$  were immobilized on the clean gold surfaces by adsorption at ocp for 60 min from  $3.0 \times 10^{-3}$  M  $\beta\text{-CD}_n$  solutions prepared in a 0.050 M phosphate buffer solution pH 7.40 followed by careful rinsing with buffer solution. The resulting surfaces are indicated as Au/ $\beta\text{-CD}_n$ , (where  $n = 1, 2$  or 7).

**Scheme 2B: Covalent attachment on gold surfaces modified with 4-MBA.** Clean gold surfaces were immersed in a fresh  $1.0 \times 10^{-3}$  M 4-MBA ethanolic solution for 30 min, followed by careful rinsing with ethanol and deionized water. The carboxylic groups of 4-MBA were activated with a 0.050 M equimolar solution of EDC and NHS (prepared in a 0.050 M phosphate buffer solution pH 7.40) for 30 min and then carefully rinsed with the buffer solution. The immobilization of the cyclodextrins was performed by amine coupling for 60 min from  $3.0 \times 10^{-3}$  M  $\beta\text{-CD}_n$  ( $n = 1, 2$  or 7) solutions prepared in a 0.050 M phosphate buffer solution at pH 7.40, followed by copiously rinsing with the phosphate buffer solution. The remaining active sites of the modified gold surface were blocked with 1 M ethanolamine (EtOH- $\text{NH}_2$ ) for 20 min and then washed with the buffer solution. The resulting surface is indicated as Au/4-MBA/ $\beta\text{-CD}_n$ .

**Scheme 2C: Covalent attachment on gold surfaces modified by electrografting of the diazonium salt (DS) obtained from 4-amino benzoic acid.** The DSs were obtained *in situ* at  $4^\circ\text{C}$  in deoxygenated aqueous solution via reduction of a fresh  $5.0 \times 10^{-3}$  M solution of the arylamine with an equimolar solution of  $\text{NaNO}_2$  prepared in 0.500 M HCl [29,30]. The electrodes were immersed in the DS solution, and the electrografting was performed by applying two consecutive cycles between 0.500 V and  $-0.400$  V at  $0.020$  Vs $^{-1}$ . The modified electrodes were sonicated for 30 seconds in deionized water. For the SPR experiments, 100  $\mu\text{L}$  of the DS solutions were transferred to the cuvette and the electrografting was performed as previously described. The solution was removed from the cuvette, and the surface was exhaustively washed with pure water and buffer solution. The  $\beta\text{-CD}_n$  were immobilized on the gold surface modified with the DS by covalent attachment following the same protocol described for the covalent attachment on gold surfaces modified with 4-MBA. The resulting surfaces are named Au/DS-4-ABA/ $\beta\text{-CD}_n$ .

### 2.4 Electrochemical characterization

Solutions of  $\text{H}_2\text{Q}$  and  $\text{K}_3\text{Fe}(\text{CN})_6$   $1.0 \times 10^{-3}$  M prepared in 0.100 M phosphate buffer pH 7.40 were used to analyze the electrochemical behavior of the bare gold and the modified surfaces by CV at  $0.100$  Vs $^{-1}$ . The results presented are representative of the average behavior observed from five independent measurements performed for each type of surface.

## 2.5. SPR experiments

To evaluate the direct adsorption of  $\beta$ -CD<sub>n</sub>, the sensorgrams were obtained at the gold substrate by recording a baseline in 0.050 M phosphate buffer solution at pH 7.40 followed by direct adsorption of  $\beta$ -CD<sub>n</sub>. To study the covalent attachment of  $\beta$ -CD<sub>n</sub> at 4-MBA or 4-ABA, the sensorgrams started by recording the baseline in 0.050 M phosphate buffer solution at pH 7.40, followed by the activation of the carboxylic groups with EDC and NHS and the adsorption of  $\beta$ -CD<sub>n</sub>. In all experiments, after each adsorption step the surfaces were copiously rinsed with the buffer solution to remove the weakly adsorbed molecules and to determine a new baseline.

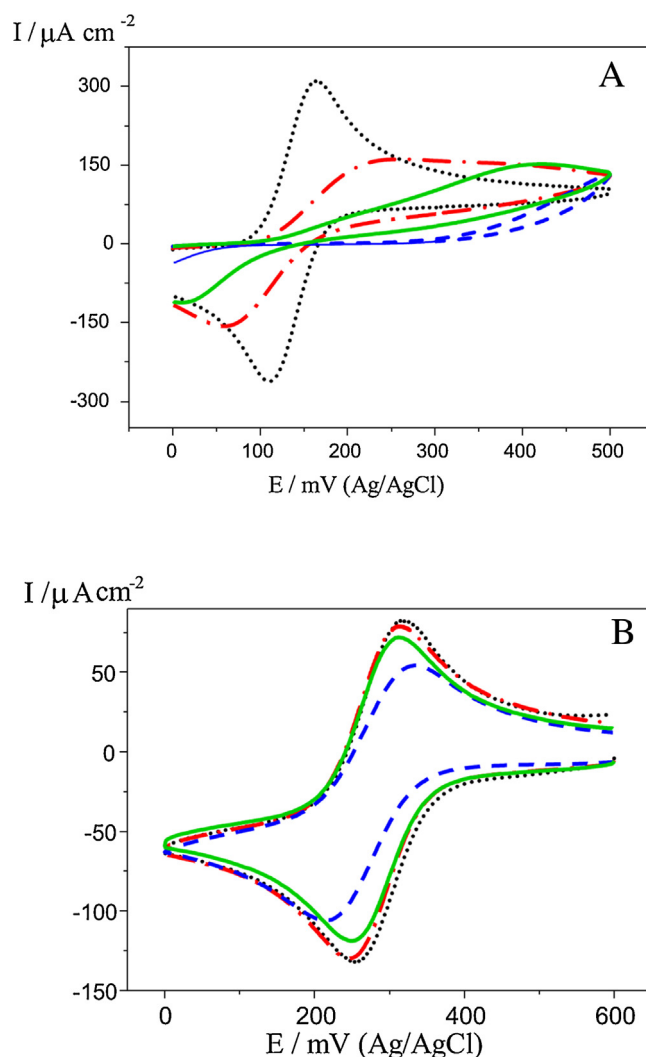
## 3 Results and discussion

We are reporting here a critical study about three different bottom-up strategies for immobilizing amino- $\beta$ -CDs on gold surfaces: the direct adsorption at bare gold surfaces, the covalent attachment at gold surfaces modified by the self-assembly of 4-MBA, and the covalent attachment at gold surfaces electrografted with DS of 4-ABA. The resulting surfaces were studied by CV using two redox probes, H<sub>2</sub>Q and [Fe(CN)<sub>6</sub>]<sup>3-</sup>. H<sub>2</sub>Q presents a quasi-reversible electrochemical behavior and follows a general mechanism with two electron transfer steps interspersed with two fast proton transfers [31]. The adsorption of H<sub>2</sub>Q at electrode surfaces decreases the reorganization energy required during the oxidation and reduction of the molecule, consequently, the kinetics of the redox process strongly depends on the adsorption of the molecule on the electrode surface (inner-sphere mechanism) [32–34]. Besides, H<sub>2</sub>Q can be incorporated into the cavity of  $\beta$ -CD forming an inclusion complex without formation of covalent bonds [26,35]. Both reasons make H<sub>2</sub>Q an appropriate redox couple to characterize the modified surfaces. [Fe(CN)<sub>6</sub>]<sup>3-</sup> has been also used to characterize surfaces modified with CDs because its size (larger than the  $\beta$ -CD cavity) and high charge density prevent the formation of inclusion complexes with  $\beta$ -CD [36,37]. Thus, variations on the electrochemical response of the molecule at the modified surface can be indirectly associated to the coverage of  $\beta$ -CD.

Surface plasmon resonance was used to determine the amount of  $\beta$ -CD immobilized at the surface. This is an optical method sensitive to the changes of the refractive index in the near vicinity of a metal surface. These changes can be associated to the refractive index difference of the medium, e.g. a change in the buffer composition or concentration; and also to the adsorption of material on the sensor surface. Thus, SPR sensing means detection of refractive index changes at the sensor surface, which in practice is related to the amount of mass deposited at the sensor surface. Direct detection is possible when the molecule under study brings about measurable refractive index changes.

### 3.1 Direct adsorption of amino- $\beta$ -CD on bare gold surfaces

Fig. 1A shows the cyclic voltammograms of  $1.0 \times 10^{-3}$  M H<sub>2</sub>Q in 0.100 M phosphate buffer solution at pH 7.40 before and after the adsorption of the amino- $\beta$ -CD (Au/ $\beta$ -CD<sub>n=1,2or7</sub>). On the bare gold electrode (dotted line), the voltammogram shows an oxidation peak at  $(169 \pm 4)$  mV, a reduction peak at  $(116 \pm 3)$  mV and a peak potential separation ( $\Delta E_p$ ) of  $(55 \pm 5)$  mV that corresponds to the hydroquinone/quinone redox reaction. A dramatic change in the electrochemical behavior of H<sub>2</sub>Q is observed at the different Au/ $\beta$ -CD<sub>n</sub> modified electrodes, with a large increment in  $\Delta E_p$  and a decrease of the peak currents density. The voltammogram obtained at Au/ $\beta$ -CD<sub>1</sub> (dashed dotted line) shows an oxidation process around 250 mV and a reduction process at 60 mV. In the case of  $\beta$ -CD<sub>7</sub> (solid line),  $\Delta E_p$  is  $(395 \pm 8)$  mV, while for  $\beta$ -CD<sub>2</sub>



**Fig. 1.** Cyclic voltammograms obtained at bare gold (---); Au/ $\beta$ -CD<sub>1</sub> (-·-·-), Au/ $\beta$ -CD<sub>2</sub> (-·-·-) and Au/ $\beta$ -CD<sub>7</sub> (—) in A)  $1.0 \times 10^{-3}$  M H<sub>2</sub>Q and B)  $1.0 \times 10^{-3}$  M K<sub>3</sub>[Fe(CN)<sub>6</sub>]. Supporting electrolyte 0.100 M phosphate buffer solution pH 7.40,  $v = 0.100$  V s<sup>-1</sup>.

(dashed line), the oxidation current increases at very high potentials, without a clear definition of a current peak.

Two contributions to the electron transfer process are possible for H<sub>2</sub>Q: reactions on the uncovered surface of the electrode, which should occur at similar potentials than at the bare electrode, and reactions through the amino- $\beta$ -CD cavities, which require higher overvoltages [26]. The incidence of each process depends on the amino- $\beta$ -CD surface coverage and the organization of the molecules at the electrode surface. At Au/ $\beta$ -CD<sub>7</sub> and Au/ $\beta$ -CD<sub>2</sub>, since the charge transfer process of H<sub>2</sub>Q takes place at elevated overvoltages together with a decrease in the current, the process is principally associated with the inclusion complex of hydroquinone in the  $\beta$ -CD cavities, while the process on uncovered surface has little contribution in the voltammetric response. In the case of Au/ $\beta$ -CD<sub>1</sub>, not so elevated overvoltages were observed, the oxidation peak potential of H<sub>2</sub>Q being observed at 81 mV higher than at bare electrode. This result suggests that, in addition to the reaction of H<sub>2</sub>Q through the cavities, the redox reaction on the uncovered surface has a slightly larger contribution in the voltammetric response as compared to those of Au/ $\beta$ -CD<sub>7</sub> and Au/ $\beta$ -CD<sub>2</sub>. Similar results were reported by Campiña et al. [26] for  $\beta$ -CDs covalently immobilized on 11-amino-1-undecanethiol self-assembled on polycrystalline gold surface, where the oxidation at higher



potential (peak at 0.7–0.8V) was associated with the guest molecules of hydroquinone reacting within the  $\beta$ -CD cavities, while the contribution of non-specific adsorption of hydroquinone appears as a shoulder at lower potentials (0.6V).

Fig. 1B depicts cyclic voltammograms performed in  $1.0 \times 10^{-3}$  M  $K_3[Fe(CN)_6]$ . The typical reversible electrochemical behavior of the redox molecule is observed on the bare gold electrode with an  $I_{pc} = (133 \pm 3) \mu A cm^{-2}$  and  $\Delta E_p = (60 \pm 2) mV$ . A slight decrease of the peak current densities, while  $\Delta E_p$  is almost constant, is evident at the Au/ $\beta$ -CD<sub>1</sub> ( $I_{pc} = (128 \pm 4) \mu A cm^{-2}$ ;  $\Delta E_p = (65 \pm 3) mV$ ), and Au/ $\beta$ -CD<sub>7</sub> ( $I_{pc} = (119 \pm 4) \mu A cm^{-2}$ ;  $\Delta E_p = (61 \pm 2) mV$ ). A more significant change was observed at Au/ $\beta$ -CD<sub>2</sub> ( $I_{pc} = (106 \pm 8) \mu A cm^{-2}$ ) with an increase in  $\Delta E_p$  up to  $(127 \pm 12) mV$ .

Considering that the size of ferricyanide is higher than the  $\beta$ -CD cavity, the current observed could be attributed to the response of the molecule through the defects or uncovered zones on the surface. But also, the adsorbed  $\beta$ -CD<sub>n</sub> molecules would form a thin layer and the electron transfer reaction of ferricyanide could take place even through the  $\beta$ -CD barrier in a way similar to that observed by Kubota et al. [38] for the redox reaction of ferricyanide through a monolayer formed by short-chain thiols. We have determined the hindrance (B) as a qualitative parameter for the layer density, following previous procedures [15] considering the ratio between the oxidation peak current measured at the modified electrode and the peak current at the bare electrode. In this way, values of 4, 11 and 20% were obtained for  $\beta$ -CD<sub>1</sub>,  $\beta$ -CD<sub>7</sub> and  $\beta$ -CD<sub>2</sub>, respectively. This tendency is in agreement with the response observed with H<sub>2</sub>Q.

It is important to remark that the electrochemical experiments give indirect information about the permeability of the redox compounds through the structure of the surface attached film, and the responses obtained correspond to the average distribution of the molecules in the surface. The selection of a molecule with an inner-sphere mechanism that forms inclusion complexes with CDs and other that is excluded from the CDs cavity results complementary. The percentage of available electrode surface is higher for  $\beta$ -CD<sub>1</sub> and the change in the electrochemical response of H<sub>2</sub>Q is less evident.

To complement the electrochemical information, the direct adsorption of  $\beta$ -CDs on gold surfaces was also evaluated by SPR (not shown). We monitored in real time the shift in the SPR angle when the gold substrate was in contact with the  $\beta$ -CD<sub>n</sub> solutions. The variations of the SPR angle,  $\Delta\theta$ , were determined after injection of the original baseline buffer; in this way the variations observed can be associated only to the adsorbed molecules. In addition, as the refractive indexes of the  $\beta$ -CD<sub>n</sub> are similar, a direct comparison of the  $\Delta\theta$  values can be performed. We determined that  $\beta$ -CD<sub>1</sub> showed only very weak and non-specific adsorption at the gold surface, being the molecules completely removed from the surface during the washing step.  $\beta$ -CD<sub>2</sub> presented a small change in the SPR angle,  $\Delta\theta = 35$  millidegrees, while  $\beta$ -CD<sub>7</sub> produced a change in the SPR angle of  $\Delta\theta = 80$  millidegrees. The small changes observed indicate that the direct adsorption of  $\beta$ -CD<sub>n</sub> produces low surface coverage, close to the sensitivity limit of the method. The values also indicate that the increase in amine groups of  $\beta$ -CD would favor the accumulation of the molecules at the surface, resulting in a highest coverage in the case of  $\beta$ -CD<sub>7</sub>.

The results obtained by the electrochemical studies can be correlated with those obtained by SPR taking into account the organization of the molecules on the modified surfaces. The increment of the amino groups in the  $\beta$ -CD could also favor the interactions between molecules and results in a heterogeneous coverage of the surface with uncovered zones and regions where the molecules are aggregated. In this sense, Huskens and co-workers have reported that the multivalent exposure of amino moieties allows stable adsorption of CDs at gold surfaces and favors the formation of multilayered structures [20].

### 3.2. Immobilization of amino- $\beta$ -CDn by covalent attachment at gold surfaces modified with 4-MBA

Fig. 2 shows the cyclic voltammograms obtained in  $1.0 \times 10^{-3}$  M H<sub>2</sub>Q (Fig. 2A) and  $1.0 \times 10^{-3}$  M ferricyanide (Fig. 2B). Fig. 2A

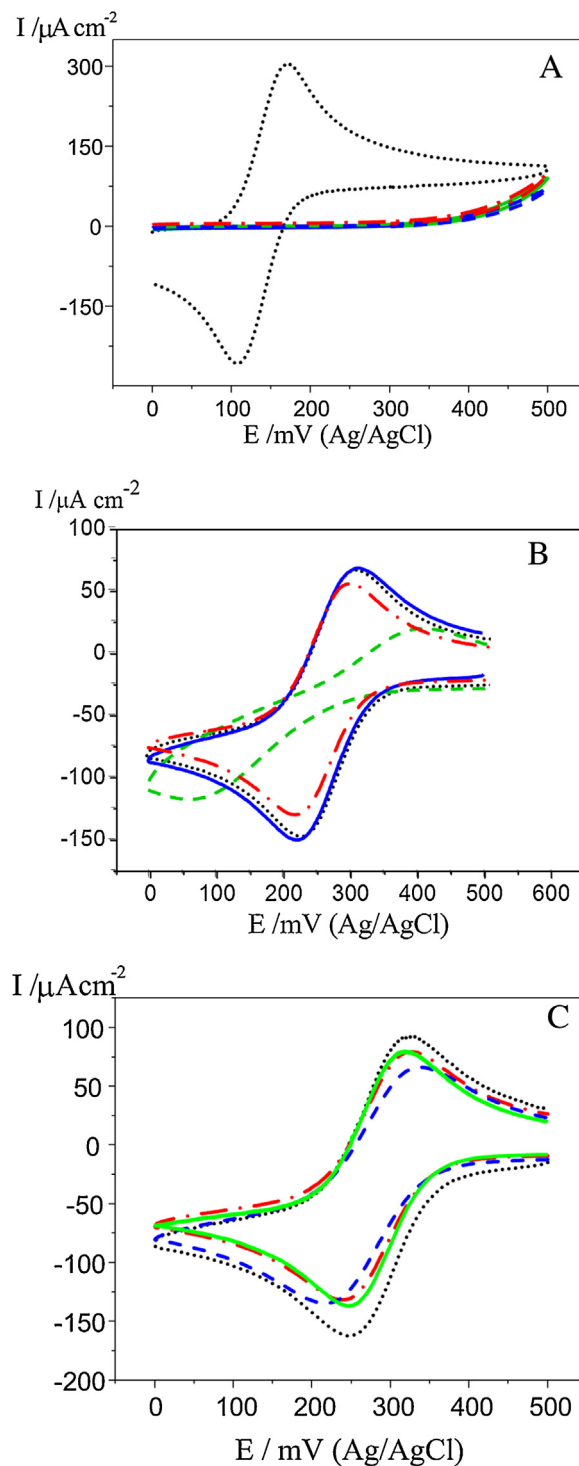


Fig. 2. Cyclic voltammograms obtained in A)  $1.0 \times 10^{-3}$  M H<sub>2</sub>Q on bare gold (....); Au/4-MBA (—), Au/4-MBA/EDC + NHS (---) and Au/4-MBA/ $\beta$ -CD<sub>1</sub> (-.-.) in 0.100 M phosphate buffer solution pH 7.4; B)  $1.0 \times 10^{-3}$  M  $K_3[Fe(CN)_6]$  in 0.100 M phosphate buffer solution pH 8.0 on bare gold (....); Au/4-MBA (—), Au/4-MBA/EDC + NHS (---) and Au/4-MBA/ $\beta$ -CD<sub>1</sub> (-.-.) and C)  $1.0 \times 10^{-3}$  M  $K_3[Fe(CN)_6]$  in 0.100 M phosphate buffer solution pH 8.0 on Au/4-MBA/EDC + NHS (....); Au/4-MBA/ $\beta$ -CD<sub>1</sub> (-.-.), Au/4-MBA/ $\beta$ -CD<sub>2</sub> (---) and Au/4-MBA/ $\beta$ -CD<sub>7</sub> (—), at  $v = 0.100 V s^{-1}$ .

displays the electrochemical response of  $H_2Q$  on different gold surfaces: Au (dotted line), Au/4-MBA (solid line), Au/4-MBA/EDC+NHS (dashed line) and Au/4-MBA/ $\beta$ -CD<sub>1</sub> (dashed dotted line). The decrease in the oxidation/reduction currents at Au/4-MBA as compared to the bare gold electrode suggests that the modification with 4-MBA forms a packed and compact structure on the gold surface that hinders the interaction of  $H_2Q$  with the surface and prevents the electrochemical reaction. As expected, after the covalent modification of the carboxylic groups with EDC+NHS and  $\beta$ -CD<sub>n</sub> the same behavior is observed.

The electrochemical response of ferricyanide at the different electrodes is shown in Fig. 2B. A lower current density ( $I_{pc} = (118 \pm 12) \mu A cm^{-2}$ ) and a higher  $\Delta E_p$  ( $380 \pm 15 mV$ ) are observed at Au/4-MBA (dashed line) compared to the bare gold electrode ( $I_{pc} = (150 \pm 4) \mu A cm^{-2}$  and  $\Delta E_p = (60 \pm 2) mV$ ; dotted line). The electrostatic repulsions between the highly negatively charged ferricyanide and the deprotonated carboxylic terminal group of 4-MBA exposed to the solution prevent the approach of the redox probe to the surface and, consequently, hinder the electron transfer process [39]. The activation of the carboxylic groups with EDC+NHS neutralizes the charge on the surface, and the voltammogram at Au/4-MBA/EDC+NHS ( $I_{pc} = (154 \pm 10) \mu A cm^{-2}$  and  $\Delta E_p = (69 \pm 5) mV$ ; solid line) becomes very similar to that obtained at the bare electrode. The covalent attachment of  $\beta$ -CD<sub>1</sub> results in a small decrease in the peak current (approximately 15%) with no changes in the  $\Delta E_p$  value (dashed dotted line). Fig. 2C compares the electrochemical response of ferricyanide on Au/4-MBA/EDC+NHS and Au/4-MBA/ $\beta$ -CD<sub>n(1,2, and 7)</sub>. When  $\beta$ -CD<sub>n</sub> were immobilized by covalent attachment, a decrease of the peak current densities of around 18% was observed in all the cases.  $\Delta E_p$  values of 83, 84 and 95 mV (with variation of ca.  $\pm 4 mV$ ) were obtained with  $\beta$ -CD<sub>7</sub>,  $\beta$ -CD<sub>1</sub> and  $\beta$ -CD<sub>2</sub>, respectively.

The covalent attachment of  $\beta$ -CD<sub>n</sub> on Au/4-MBA was also studied by SPR. The sensorgram obtained for  $\beta$ -CD<sub>7</sub> is shown in Fig. 3A. When a  $\beta$ -CD<sub>7</sub> solution is injected into the cuvette a rapid increase in the resonance angle is observed, achieving a stationary signal after 45 min. To remove the weakly bound molecules and to determine the new baseline, the surface was washed three times with 0.050 M phosphate buffer solution at pH 7.40 until a stationary response was obtained. The variation of the SPR angle,  $\Delta\theta_{SPR}$ , was determined from the difference in the signal recorded in the buffer before and after the interaction of  $\beta$ -CD<sub>7</sub> with the surface. Equivalent experiments, Fig. 3B, were performed with  $\beta$ -CD<sub>1</sub> and  $\beta$ -CD<sub>2</sub>. From the results, it is evident that the adsorption of  $\beta$ -CD<sub>1</sub> is not detectable under the working conditions because the same SPR angle was obtained before and after the interaction of the surface with the molecule. ( $\Delta\theta_{SPR} = 0$ ). In the case of  $\beta$ -CD<sub>2</sub> and  $\beta$ -CD<sub>7</sub> the values of  $\Delta\theta_{SPR}$  were  $(73 \pm 8)$  and  $(150 \pm 53)$  millidegrees, respectively, which we associate to the immobilization of  $\beta$ -CD<sub>n</sub> by covalent attachment with the carboxylic groups. At the relatively high concentration of  $\beta$ -CD<sub>n</sub> used in the experiments, mass transport limitation is avoided, and the amount of  $\beta$ -CD attached to the surface only depends on the interactions with the anchoring groups, and thus, it can be regarded as a measurement of the ability to form covalent bonds with the modified substrate. The values of  $\Delta\theta_{SPR}$  indicate that the surface coverage is higher for  $\beta$ -CD<sub>7</sub>. Despite the increase in the amount of adsorbed molecules with the number of amine groups linked to  $\beta$ -CD, this relationship is not linear. This could be related to steric effects preventing the formation of the chemical bonds between closer molecules.

### 3.3. Immobilization of $\beta$ -CD<sub>n</sub> by covalent attachment at gold surfaces modified by electrografting of diazonium salt obtained from 4-ABA

Fig. 4A shows the electrochemical response of  $H_2Q$  on Au (dotted line), Au/DS-4-ABA (solid line), Au/DS-4-ABA/EDC+NHS

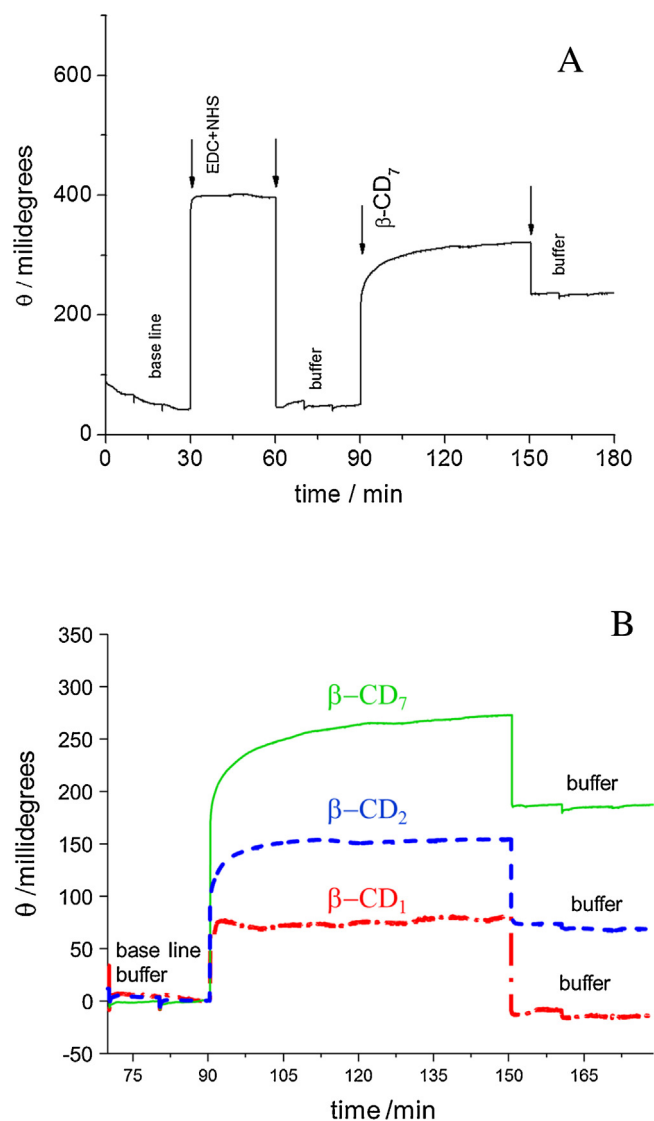
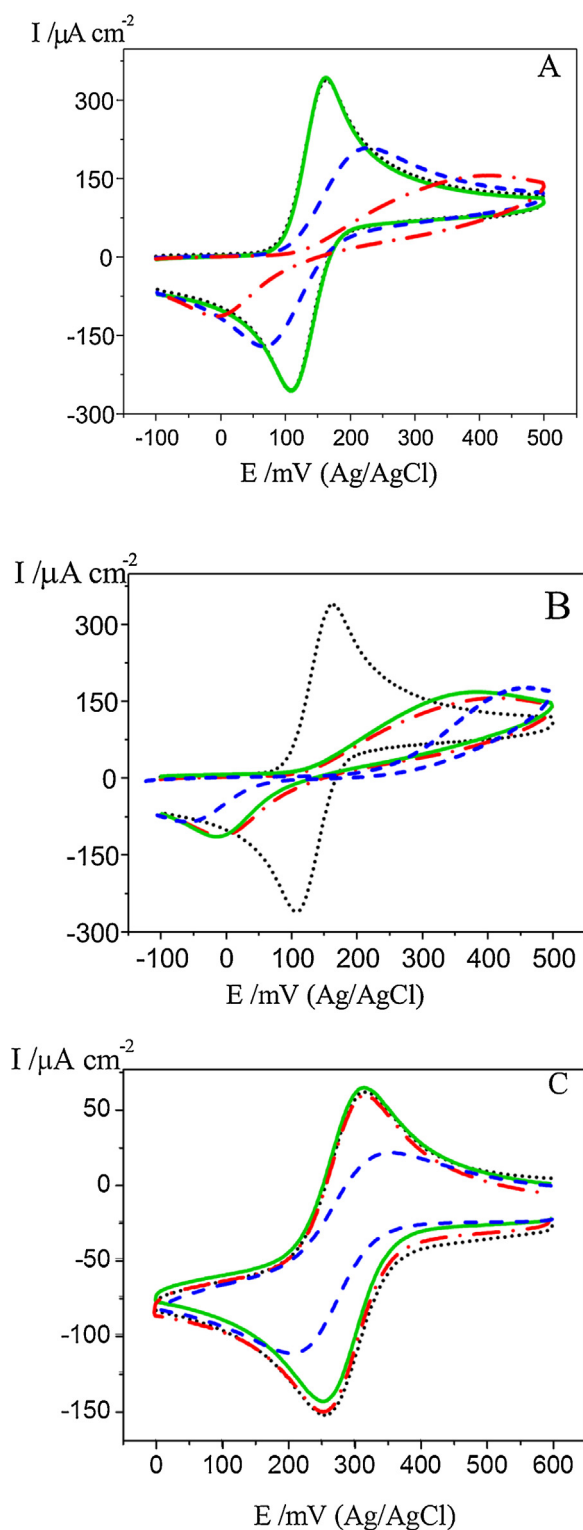


Fig. 3. Sensorgram obtained during the adsorption of  $\beta$ -CD<sub>7</sub> on Au/4-MBA. Arrows indicate the addition of 0.050 M phosphate buffer solution at pH 7.40,  $50.0 \times 10^{-3} M$  equimolar solution of EDC+NHS and  $3.0 mg mL^{-1}$   $\beta$ -CD<sub>7</sub> to the cuvette. B) Overlapped sensorgrams obtained during the adsorption of  $\beta$ -CD<sub>1</sub> (---),  $\beta$ -CD<sub>2</sub> (---) and  $\beta$ -CD<sub>7</sub> (—).

(dashed line) and Au/DS-4-ABA/ $\beta$ -CD<sub>1</sub> (dashed dotted line). The immobilization of 4-ABA does not block the electrochemical response of  $H_2Q$  (solid line), indicating that its structure presents pores, holes and defects opposite to the well-packed layer of the 4-MBA thiol analog. According to the literature [39], the three-dimensional porous structure of this layer allows the diffusion through the channels of small molecules such as  $H_2Q$  which are able to reach the bare gold surface. The activation by EDC+NHS (dashed line) partially blocks the access of  $H_2Q$  to the surface,  $\Delta E_p$  increases up to  $(162 \pm 12) mV$  and the oxidation peak current decreases around 40%, most likely due to a change in the hydrophobic character and the occlusion of some pores in the layer. The covalent attachment of  $\beta$ -CD<sub>1</sub> (dashed dotted line) produces a significant increment of  $\Delta E_p$  ( $404 \pm 12$ ) mV. Fig. 4B shows the electrochemical response of  $H_2Q$  on the modified surfaces with  $\beta$ -CD<sub>n</sub>. Similar behavior was observed with all of them:  $\Delta E_p$  of  $(398 \pm 12) mV$  was registered for Au/DS-4-ABA/ $\beta$ -CD<sub>7</sub>, while for Au/DS-4-ABA/ $\beta$ -CD<sub>2</sub> this increment was even higher being  $\Delta E_p = (507 \pm 18) mV$  (solid and dashed lines, respectively). The electrochemical response of ferricyanide on these



**Fig. 4.** Cyclic voltammograms obtained in  $1.0 \times 10^{-3}$  M  $\text{H}_2\text{Q}$  on A) bare gold (.....); Au/DS-4-ABA (—), Au/DS-4-ABA/EDC+NHS (---) and Au/DS-4-ABA/ $\beta$ -CD<sub>1</sub> (-.-.);  $1.0 \times 10^{-3}$  M  $\text{H}_2\text{Q}$  on B) Au/DS-4-ABA (.....); Au/DS-4-ABA/ $\beta$ -CD<sub>1</sub> (-.-.), Au/DS-4-ABA/ $\beta$ -CD<sub>2</sub> (---) and Au/DS-4-ABA/ $\beta$ -CD<sub>7</sub> (—). C) Electrochemical response in  $1.0 \times 10^{-3}$  M  $\text{K}_3[\text{Fe}(\text{CN})_6]$  obtained on Au/DS-4-ABA/EDC+NHS (.....); Au/DS-4-ABA/ $\beta$ -CD<sub>1</sub> (-.-.), Au/DS-4-ABA/ $\beta$ -CD<sub>2</sub> (---) and Au/DS-4-ABA/ $\beta$ -CD<sub>7</sub> (—). Supporting electrolyte: 0.100 M phosphate buffer solution pH 7.40. Scan rate 0.100  $\text{V s}^{-1}$ .

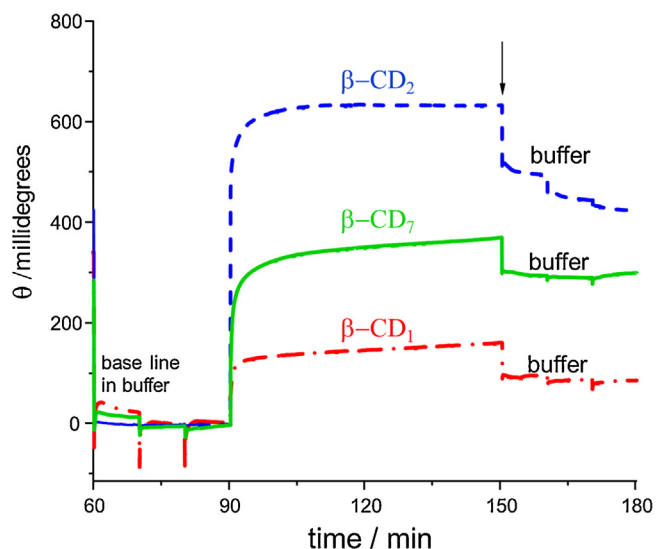
structures was also analyzed (Fig. 4C). At Au/DS-4-ABA/ $\beta$ -CD<sub>1</sub> (dashed dotted line) and Au/DS-4-ABA/ $\beta$ -CD<sub>7</sub> (solid line) there are only small changes in the voltammogram while a decrease in

current density of approximately 30% and an increase in the  $\Delta E_p$  up to  $(148 \pm 22)$  mV were observed for Au/DS-4-ABA/ $\beta$ -CD<sub>2</sub> (dashed line) in comparison with the results obtained at the Au/DS-4-ABA/EDC+NHS electrode. These results can be rationalized considering that the attached film is porous allowing the  $[\text{Fe}(\text{CN})_6]^{3-}$  and  $\text{H}_2\text{Q}$  electron transfer reactions at the uncovered zones of the Au electrode that depends on the percentage of the available electrode surface. From the results obtained with both redox probes, it could be concluded that this percentage is lower for  $\beta$ -CD<sub>2</sub> means that higher number of these molecules are attached covalently to the Au/DS-4-ABA/EDC+NHS surface. To confirm this point, a comparison of the amount of molecules immobilized by this method was evaluated by SPR.

Fig. 5 shows the sensorgrams obtained during the covalent attachment of  $\beta$ -CD<sub>n</sub> on gold surfaces modified by electrografting of the DS obtained from 4-ABA.  $\Delta\theta_{\text{SPR}}$  values of  $(77 \pm 8)$ ,  $(4.0 \pm 0.2) \times 10^2$  and  $(2.9 \pm 0.1) \times 10^2$  millidegrees were obtained for  $\beta$ -CD<sub>1</sub> (dashed dotted line),  $\beta$ -CD<sub>2</sub> (dashed line) and  $\beta$ -CD<sub>7</sub> (solid line), respectively. These values correspond to the average of three independent measurements for each  $\beta$ -CD<sub>n</sub>. Taking into account the fact that the molecular weights of the three  $\beta$ -CD<sub>n</sub> are similar, the amount of adsorbed  $\beta$ -CD<sub>2</sub> is 1.4 and 5.2 times higher than those of  $\beta$ -CD<sub>7</sub> and  $\beta$ -CD<sub>1</sub>, respectively. These results are in good agreement with the electrochemical results previously described.

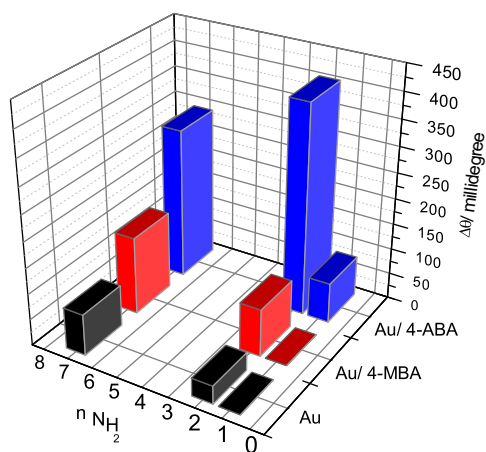
Conclusive results about the quantity of  $\beta$ -CD<sub>n</sub> on gold surfaces can be obtained comparing the SPR results. Fig. 6 compares the variations in  $\Delta\theta_{\text{SPR}}$  due to the immobilization of  $\beta$ -CD<sub>n</sub> on gold surfaces using the different strategies. The largest changes are observed for the surfaces modified via electrografting of the diazonium salt. Therefore, we can conclude that more  $\beta$ -CD<sub>n</sub> is immobilized in this supramolecular architecture than in the well-ordered and packed architecture of the Au/4-MBA. This difference is related to the number of anchoring points in the sublayer. It is well-known that the electrografting of DS produces multilayered porous layers with a large amount of benzoic acid groups available for the subsequent covalent modification [40,41].

The direct adsorption results in the lowest amount of  $\beta$ -CD<sub>n</sub> without significant control of the adsorptive process. This result can be interpreted considering the weak interaction of the amine groups with the gold surface [20]. Independently of the method



**Fig. 5.** Overlapped sensorgrams obtained during the adsorption of  $\beta$ -CD<sub>1</sub> (-.-.),  $\beta$ -CD<sub>2</sub> (---) and  $\beta$ -CD<sub>7</sub> (—) on gold surfaces modified by electrografting of 4-ABA diazonium salt. Arrows indicate the addition of 0.050 M phosphate buffer solution at pH 7.40 to the cuvette.





**Fig. 6.** The variation of the SPR angle,  $\Delta\theta_{\text{SPR}}$ , as a function of the number of amine groups on  $\beta$ -CD for each immobilization method: direct adsorption on bare gold, amide bond formation with a 4-MBA self-assembled layer and electrografted diazonium salt from 4-ABA.

used, the amounts of immobilized  $\beta$ -CD<sub>2</sub> and  $\beta$ -CD<sub>7</sub> are always higher than that of  $\beta$ -CD<sub>1</sub>. The presence of two or more amino terminal groups on the primary rim of  $\beta$ -CD<sub>n</sub> favors chemical coupling, although the steric hindrance slightly unfavors the immobilization of  $\beta$ -CD<sub>7</sub>.

#### 4 Conclusions

Amino- $\beta$ -CDs have been successfully immobilized on gold electrodes via amide bond formation with the carboxylic groups of 4-MBA and the electrografted diazonium salt of the analogous aromatic amine 4-ABA. The direct interaction between amino- $\beta$ -CD and bare gold produces non-specific and weak adsorption of the  $\beta$ -CD and partial coverage of the surface. The electrochemical and SPR results show that the surface coverage depends on the number of amine groups on the primary rim of  $\beta$ -CD and the method of the electrode modification. In the case of the well-ordered and packed thiolated layer, the surface coverage is proportional to the number of amine groups, while the disordered and porous structure of the electrografted diazonium layer allows higher surface coverage; however, the steric hindrance is more favourable for the  $\beta$ -CD with two anchoring amino groups.

As we have shown, the results presented in this work offer an innovative alternative to immobilize  $\beta$ -CD on gold surfaces without the requirement of synthesizing thiol or diazonium-modified cyclodextrins. These important advantages of the supramolecular architectures proposed here represent a significant contribution to the field of host-guest interactions opening the door to future analytical devices.

#### Acknowledgements

The authors thank FONDECYT Grant 1151329 (Chile), CONICET, SECYT-UNC and Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT) (Argentina) and Argentina-Chile International Collaboration Grant CONICYT/MINCYT 2013 (PCCI130050, CH/13/03) for the financial support.

#### References

- [1] J. Szejtli, Past, present and future of cyclodextrin research, *Pure App. Chem.* 76 (2004) 1825.
- [2] S.V. Kurkov, T. Loftsson, *Cyclodextrins*, *Int. J. Pharm.* 453 (2013) 167.
- [3] V. Perchyonok, T. Oberholzer, *Cyclodextrins as Oral Drug Carrier Molecular Devices: Origins Reasons and In-vitro Model Applications*, *Curr. Org. Chem.* 16 (2012) 2365.
- [4] M.R. Sambrook, S. Notman, *Supramolecular chemistry and chemical warfare agents: from fundamentals of recognition to catalysis and sensing*, *Chem. Soc. Rev.* 42 (2013) 9251.
- [5] C.A. Kozłowski, T. Girek, W. Walkowiak, J.J. Kozioł, Application of hydrophobic  $\beta$ -cyclodextrin polymer in separation of metal ions by plasticized membranes, *Sep. Purif. Technol.* 46 (2005) 136.
- [6] M.A. Aroon, A.F. Ismail, T. Matsuura, Application of hydrophobic  $\beta$ -cyclodextrin polymer in separation of metal ions by plasticized membranes, *Sep. Purif. Technol.* 115 (2013) 39.
- [7] A. Hashidzume, H. Yamaguchi, A. Harada, *Molecular Machines and Motors: Recent Advances and Perspectives*, in: Credi, Alberto, Silvi, Serena, Venturi, Margherita (Eds.), *Colección: Topics in Current Chemistry*, 354, Páginas, 2014, pp. 71.
- [8] F. Niess, V. Duplan, J.-P. Sauvage, *Molecular Muscles From Species in Solution to Materials and Devices*, *Chem. Lett.* 43 (2014) 964.
- [9] J.C. Easton, *Cyclodextrin-based catalysts and molecular reactors*, *Pure Appl. Chem.* 77 (2005) 1865.
- [10] P. Shahgaldian, U. Pielas, *Cyclodextrin, Derivatives as Chiral Supramolecular Receptors for Enantioselective Sensing*, *Sensors* 6 (2006) 593.
- [11] A.E. Kaifer, M. Gomez-Kaifer, *Supramolecular Electrochemistry: Self-Assembled Monolayers*, Wiley-VCH, Weinheim, 1999 chapter 15.
- [12] S.-J. Choi, S.-M. Park, *Electrochemical Characterization of Nanosized Electrode Arrays Prepared from Nanoporous Self-Assembled Monolayers*, *Bull. Korean Chem. Soc.* 23 (2002) 699.
- [13] M.T. Rojas, R. Koniger, J.F. Stoddart, A.E. Kaifer, *Supported monolayers containing preformed binding sites. Synthesis and interfacial binding properties of a thiolated beta-cyclodextrin derivative*, *J. Am. Chem. Soc.* 117 (1995) 336.
- [14] Y. Maeda, T. Fukuda, H. Yamamoto, H. Kitano, *Regio- and Stereoselective Complexation by a Self-Assembled Monolayer of Thiolated Cyclodextrin on a Gold Electrode*, *Langmuir* 13 (1997) 4187.
- [15] S. Bollo, C. Yáñez, J. Sturm, L. Núñez-Vergara, J.A. Squella, *Cyclic Voltammetric and Scanning Electrochemical Microscopic Study of Thiolated  $\beta$ -Cyclodextrin Adsorbed on a Gold Electrode*, *Langmuir* 19 (2003) 3365.
- [16] I. Carrillo, C. Quintana, A.M. Esteve, L. Hernández, P. Hernández, *Self-Assembled Submonolayer of and  $\beta$ -Cyclodextrins on Gold Electrode for the Selective Determination of 4-Aminobiphenyl*, *Electroanal.* 23 (2011) 2862.
- [17] M.D. Porter, T.B. Bright, D.L. Allara, C.E.D. Chidsey, *Spontaneously organized molecular assemblies. 4. Structural characterization of n-alkyl thiol monolayers on gold by optical ellipsometry, infrared spectroscopy, and electrochemistry*, *J. Am. Chem. Soc.* 109 (1987) 3559.
- [18] G. Nelles, M. Weisser, R. Back, P. Wohlhart, G. Wenz, S. Mittler-Neher, *Controlled Orientation of Cyclodextrin Derivatives Immobilized on Gold Surfaces*, *J. Am. Chem. Soc.* 118 (1996) 5039.
- [19] M.W.J. Beulen, J. Bügler, B. Lammerink, F.A.J. Geurts, E.M.E.F. Biemond, K.G.C. van Leerdam, F.C.J.M. van Veggel, J.F.J. Engbersen, D.N. Reinhoudt, *Self-Assembled Monolayers of Heptapodant  $\beta$ -Cyclodextrins on Gold*, *Langmuir* 14 (1998) 6424.
- [20] A. Méndez-Ardoy, T. Steentjes, T. Kudernac, J. Huskens, *Self-Assembled Monolayers on Gold of  $\beta$ -Cyclodextrin Adsorbates with Different Anchoring Groups*, *Langmuir* 30 (2014) 3467.
- [21] M. Khoshroo, A. Rostami, *Characterization of the organic molecules deposited at gold surface by the electrochemical reaction of diazonium salts*, *J. Electroanal. Chem.* 647 (2010) 117.
- [22] X. Wang, H. Fan, F. Zhang, Y. Qi, W. Qiu, F. Yang, J. Tang, P. He, *Synthesis of a  $\beta$ -cyclodextrin derivative and its molecular recognition behavior on modified glassy carbon electrode by diazotization*, *Tetrahedron* 66 (2010) 7815.
- [23] J. Stensgaard Diget, H. Nørgaard Petersen, H. Schaarup-Jensen, A. Ulrik Sørensen, T. Terndrup Nielsen, S. Utrup Pedersen, K. Daasbjerg, K. Lambertsen Larsen, M. Hinge, *Synthesis of  $\beta$ -Cyclodextrin Diazonium Salts and Electrochemical Immobilization onto Glassy Carbon and Gold Surfaces*, *Langmuir* 28 (2012) 16828.
- [24] M. Lahav, K.T. Ranjit, E. Katz, I. Willner, *A b-amino-cyclodextrin monolayer-modified Au-electrode: A command surface for the amperometric and microgravimetric transduction of optical signals recorded by a photoisomerizable bipyridinium-azobenzene dyad*, *Chem. Commun.* (1997) 259.
- [25] E. Katz, *A chemically modified electrode capable of a spontaneous immobilization of amino compounds due to its functionalization with succinimidyl groups*, *J. Electroanal. Chem.* 291 (1990) 257.
- [26] J.M. Campiña, A. Martins, F. Silva, *Immobilization of  $\beta$ -cyclodextrin on gold surfaces by chemical derivatization of an 11-amino-1-undecanethiol self-assembled monolayer*, *Electrochim. Acta* 55 (2009) 90.
- [27] X. Duan, N.K. Rajan, D.A. Routenberg, J. Huskens, M.A. Reed, *Regenerative Electronic Biosensors Using Supramolecular Approaches*, *ACS Nano* 7 (2013) 4014.
- [28] R.F. Carvalho, R.S. Freire, L.T. Kubota, *Polycrystalline Gold Electrodes: A Comparative Study of Pretreatment Procedures Used for Cleaning and Thiol Self-Assembly Monolayer Formation*, *Electroanal.* 17 (2005) 1251.
- [29] A.M. Nowicka, M. Fau, A. Kowalczyk, M. Strawski, Z. Stojek, *Electrografting of carboxyphenyl thin layer onto gold for DNA and enzyme immobilization*, *Electrochim. Acta* 126 (2014) 11.



- [30] J. Pinson, F. Podvorica, Attachment of organic layers to conductive or semiconductive surfaces by reduction of diazonium salts, *Chem. Soc. Rev.* 34 (2005) 429.
- [31] E. Laviron, Electrochemical reactions with protonations at equilibrium: Part X. The kinetics of the p-benzoquinone/hydroquinone couple on a platinum electrode, *J. Electroanal. Chem.* 164 (1984) 213.
- [32] G.E. Cabanis, A.A. Diamantis, W.R. Murphy Jr., R.W. Linton, T.J. Meyer, Electrocatalysis of proton-coupled electron-transfer reactions at glassy carbon electrodes, *J. Am. Chem. Soc.* 107 (1985) 1845.
- [33] S.Hunt DuVall, R.L. McCreery, Control of Catechol and Hydroquinone Electron-Transfer Kinetics on Native and Modified Glassy Carbon Electrodes, *Anal. Chem.* 71 (1999) 4594.
- [34] K.-H. Kung, M.B. McBride, Electron Transfer Processes Between Hydroquinone and Iron Oxides, *Clay Clay Miner.* 36 (1988) 303.
- [35] H. Endo, T. Nakaji-Hirabayashi, S. Morokoshi, M. Gemmei-Ide, H. Kitano, Orientational Effect of Surface-Confined Cyclodextrin on the Inclusion of Bisphenols, *Langmuir* 21 (2005) 1314.
- [36] L. Zheng, S. Wu, X. Lin, L. Nie, L. Rui, Preparation and Characterization of a Novel  $\beta$ -Cyclodextrin Modified Poly(N-acetylaniline) Film, *Macromolecules* 35 (2002) 6174.
- [37] M. Frasconi, F. Mazzei, Electrochemical and surface plasmon resonance characterization of  $\beta$ -cyclodextrin-based self-assembled monolayers and evaluation of their inclusion complexes with glucocorticoids, *Nanotechnology* 20 (2009) 285502.
- [38] R.K. Mendes, R.S. Freire, C.P. Fonseca, S. Neves, L.T. Kubota, Characterization of Self-Assembled Thiols Monolayers on Gold Surface by Electrochemical Impedance Spectroscopy, *J. Braz. Chem. Soc.* 15 (2004) 849.
- [39] M.G. Paulik, P.A. Brooksby, A.D. Abell, A.J. Downard, Grafting Aryl Diazonium Cations to Polycrystalline Gold: Insights into Film Structure Using Gold Oxide Reduction Redox Probe Electrochemistry, and Contact Angle Behavior, *J. Phys. Chem.* 111 (2007) 7808.
- [40] P.A. Brooksby, A.J. Downard, Multilayer Nitroazobenzene Films Covalently Attached to Carbon An AFM and Electrochemical Study, *J. Phys. Chem. B* 109 (2005) 8791.
- [41] J.K. Kariuki, M.T. McDermott, Nucleation and Growth of Functionalized Aryl Films on Graphite Electrodes, *Langmuir* 15 (1999) 6534.