Cyclic Voltammetric and Scanning Electrochemical Microscopic Study of Thiolated β-Cyclodextrin Adsorbed on a Gold Electrode

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Potassium ferricyanide ($[Fe(CN)_6]^{3-}$) reduction and ferrocenemethanol (Fc-OH) oxidation at a β-cyclodextrin (β-CD)-modified gold electrode were investigated by cyclic voltammetry (CV) and scanning electrochemical microscopy (SECM) in phosphate buffer pH 7. CV and SECM experiments demonstrated that the surface of the modified electrode represents an insulating substrate for ferricyanide and a conductor substrate for Fc-OH. This difference is explained by the fact that Fc-OH can enter into the β-CD cavity forming an inclusion complex; on the other hand, ferricyanide is larger than the cavity of β-CD and so it cannot form an inclusion complex. Using the high sensitivity of the electron transfer of ferricyanide to the modification of the gold surface with β-CD, we selected this reaction as a probe to study the different modification stages at this modified electrode. When the electrode was partially modified, it was possible to obtain an adequate microscopic discrimination by using approach curves at different tip positions showing a nonhomogeneous surface. SECM images obtained from bare and partially and totally modified electrodes show very good resolution with different topography or null according to the extent of modification. When the modification was total, a homogeneous current with a regular topography was found, but when the modification was intermediate the topography was very irregular. To our knowledge, this is the first electrochemical study of a thiolated β-CD layer adsorbed on a gold electrode by SECM, and taken together, these results demonstrate that while the CV experiments give overall information about the studied surface, the SECM experiments permit microscopic characterization of the layer.

1. Introduction

The ability of cyclodextrins (CDs) to form inclusion complexes makes possible their use in the pharmaceutical industry, food technology, and agriculture. Many of the potential analytical applications of CDs require immobilization on a surface. Ferancová and Labuda[1] published a review describing the complexation abilities and analytical applications of working electrodes with attached CDs. A convenient strategy is modifying CDs with organosulfur compounds, which bind strongly to gold surfaces. Synthesis of thiolated CDs and their absorption on gold and silver have been previously described,[2,3] and voltammetric responsive sensors using lipoyl-β-CD have been studied for detection of electroinactive organic species.[4]

In a first intent to improve the analytical determination of some drugs, we have tested a carbon paste electrode modified with β-cyclodextrin (β-CD).[5] The immobilization of the β-CD on the carbon paste leads to a modification of the electrode surface that caused a significant increase in the peak current of the electroactive species due to the formation of an inclusion complex between β-CD and the drug. In a next step, we are interested to explore the immobilization over a solid surface better than immobilization in a mixture with carbon paste. Consequently, the well-known modification of gold electrodes with thiolated β-CD is our current objective. Furthermore, the possibility of obtaining highly organized layers of thiolated β-CD is also very attractive for the study of the electrode/solution interface and electrochemical sensors.

In the past 10 years, a new form to do electrochemical experiments through scanning electrochemical microscopy, SECM, has been extensively described.[6–11] One of the applications of the SECM technique is to use the ultramicroelectrode tip to obtain images of different substrate surfaces immersed in electrolyte solutions with about micrometer resolution. It can also be used to distinguish regions of different conductivity or electrochemical activity at high resolution. In addition to its imaging capability, SECM is an electrochemical tool that can be used to study electrode reactions and homogeneous reactions of tip-generated species that occur in the small gap between tip and substrate. In the most common form of the SECM experiment, the feedback mode, the tip is used to generate, via electrolysis, a reduced or oxidized mediator species. Far from the substrate, the electrolytic current assumes the steady-state value characteristic of ultramicroelectrodes. However, as the tip is brought close to a surface, the tip current is perturbed. This perturbation is different for an insulating and for a conducting surface.

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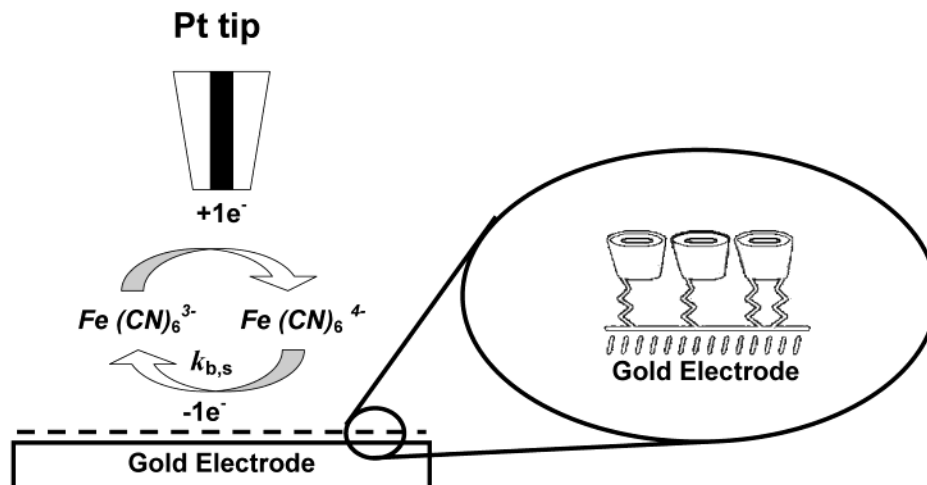(6) Kwak, J.; Bard, A. J. *Anal. Chem.* **1989**, *61*, 1221–1227.

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Figure 1. Schematic diagram for the study of modified and bare gold electrodes by SECM.

Although the modification of gold electrodes with thiolated β-CD is a known matter, no SECM analysis of this modified surface was studied previously. In this work, we are focused to study the modification that a gold electrode suffers when it is exposed to a thiolated β-CD derivative. In particular, the influences of the adsorption time and the preconditioning potential on the gold surface will be examined. For this purpose, potassium ferricyanide reduction and ferrocenemethanol (Fc-OH) oxidation at a β-CD-modified gold electrode were investigated by using both cyclic voltammetry (CV) and SECM.

With the SECM experiments, we hope to find an electrochemical microscopic view of the layer, that is not possible to obtain with conventional techniques.

2. Experimental Section

2.1. Materials. β-CD and Fc-OH were purchased from Aldrich; potassium ferricyanide, potassium dihydrogen phosphate, and sodium phosphate dibasic anhydrous were purchased from Merck. All the other reagents were of analytical grade or better.

The buffer solutions were prepared mixing solution A and solution B to a certain pH value (solution A, 0.1 M KH_2PO_4; solution B, 0.1 M Na_2HPO_4). All aqueous solutions were prepared with Milli-Q water (Millipore Co., USA).

2.2. Synthesis of Cyclodextrinthiol (CD-SH). The synthesis of the CD-SH was performed by one of us according to a well-known described methodology.[2] First, five of the seven primary hydroxyl groups of β-CD were tosylated using *p*-toluenesulfonyl chloride. Later a nucleophilic substitution with 1,6-hexanedithiol produced a multithiolated penta[6-deoxy-6-[(mercaptohexamethylene)thio]-β-cyclodextrin (CD-SH). All the intermediates and the final product were characterized by ^1H NMR.

2.3. Apparatus. The SECM and cyclic voltammetric experiments were carried out with a CHI 900 setup (CH Instruments Inc., USA). A 10 μm diameter Pt electrode was supplied by CHI (CH Instruments Inc.) and served as the SECM tip. A 0.5 mm diameter Pt wire and a Ag|AgCl KCl saturated electrode were used as counter and reference electrodes, respectively.

Before each experiment, the tip was polished with 0.3 and 0.05 μm alumina and then rinsed with water. All the experiments were carried out at room temperature (25 $\pm$ 2 °C), and the solutions were purged with pure argon for 5 min before each run.

2.4. Electrode Modification. A 3 mm diameter gold electrode (Bioanalytical Systems, USA) was polished with alumina powder (diameter, 0.3 and 0.05 μm) and rinsed with pure water. The modified electrode was obtained as was already described[12] by immersion of the gold electrode in an ethanolic solution of CD-SH for different times at room temperature (25 $\pm$ 2 °C). The modified electrode was removed from the solution and then rinsed

with absolute ethanol and water respectively before use for CV or SECM experiments.

2.5. SECM Experiments. Figure 1 shows the schematic diagram of the electrochemical cell and the setup used to investigate the modification of the gold electrode surface. Here the gold electrode was used as the substrate in SECM experiments.

The feedback mode is the main quantitative operation mode of SECM.[7,13] When the tip is far from the substrate and a potential is applied, the steady-state current, $i_{T,\infty}$, is given by

$$i_{T,\infty} = 4nFDCa$$

where F is the Faraday constant, n is the number of electrons transferred in the tip reaction, D is the diffusion coefficient of electroactive species, C is the bulk concentration of the species, and a is the tip radius. In the positive feedback results, a higher tip current is observed ($i_T > i_{T,\infty}$) when the tip is closer to the substrate, meaning that the substrate is conductive, whereas in the negative feedback results, a lower tip current is observed ($i_T < i_{T,\infty}$) when the tip is closer to the substrate, meaning that the substrate is insulating.

SECM results are presented in the dimensionless form of I_T-(L) versus L (where $I_T(L) = i_T/i_{T,\infty}$, called normalized current; $L = d/a$, and d is the distance between the tip and the substrate) and are independent of disk diameter, diffusion coefficient, and solute concentration.[14]

On the other hand, there are numerical approximations that correlated the normalized tip current and L, which permits to us calculate the distance value d.[6,7]

3. Results and Discussion

3.1. Modification of the Gold Electrode with CD-SH and Its Characterization by Cyclic Voltammetry and SECM. The Au electrode was modified according to the procedure described previously. The CD-SH was covalently bound to the gold surface via chemisorption of thiol groups to gold.

The adsorbed CD-SH can form selective barriers to inhibit the transport of species, which are incapable of complexing with β-CD, but meanwhile have the characteristic of selective transport with the host−guest interactions between the β-CD host and various organic guest species.[15] To obtain experimental evidence for the selectivity of the barrier, cyclic voltammetric experiments using two electroactive probes such as ferricyanide and ferrocenemethanol (Fc-OH) were carried out.

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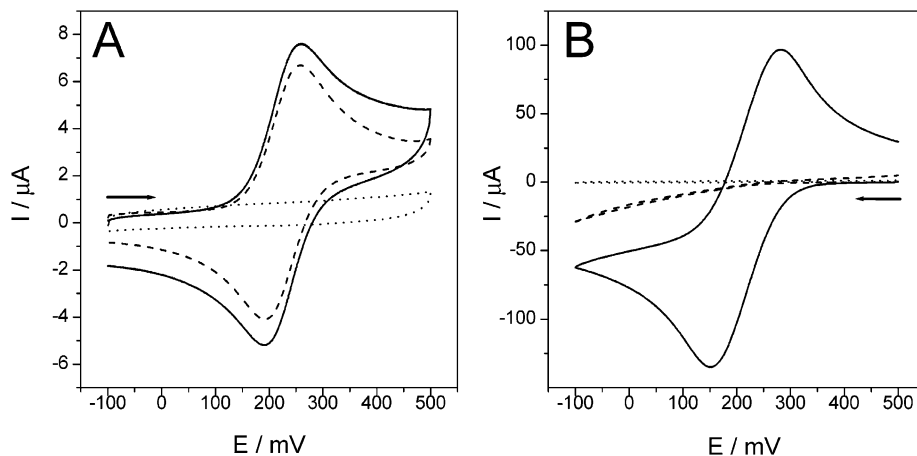
Figure 2. Cyclic voltammograms obtained for (A) 0.5 mM Fc-OH in 0.1 M phosphate buffer (pH 7.0) and (B) 0.01 M ferricyanide in 0.1 M phosphate buffer (pH 7.0) at a bare gold electrode (solid lines) and a CD-SH-modified gold electrode (dashed lines). Dotted curves are cyclic voltammograms of 0.1 M phosphate buffer (pH 7.0) at the modified CD-SH electrode. Sweep rate = 0.1 V s^{-1}.
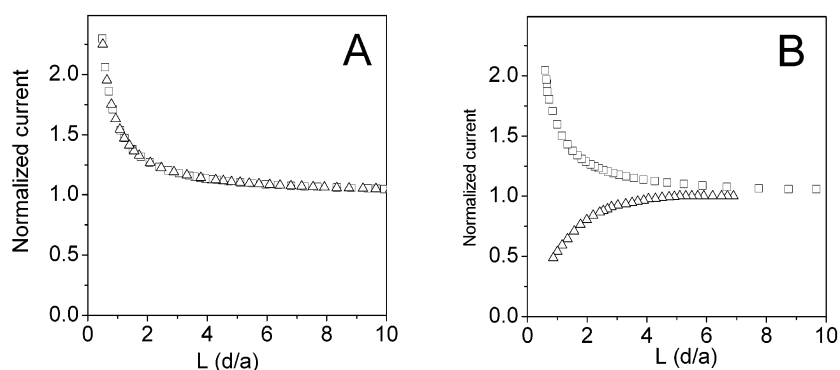
Figure 3. Normalized tip current−distance curves for the tip approaching the bare (open square) and the CD-SH modified (open triangle) gold electrode: (A) 0.5 mM Fc-OH in 0.1 M phosphate buffer (pH 7.0) and (B) 0.01 M ferricyanide in 0.1 M phosphate buffer (pH 7.0). E_{tip} = 500 mV and E_s = −1000 mV for Fc-OH, and E_{tip} = −100 mV and E_s = 500 mV for ferricyanide experiments. a = 5 μm (Pt tip radius).

Figure 2 shows the cyclic voltammogram of Fc-OH (A) and ferricyanide (B) at the CD-SH Au modified and bare Au electrodes in phosphate buffer pH 7.0. The Fc-OH molecule, complexing with β-CD as a guest, can enter into the β-CD cavity and can electrochemically react through the alkyl chain linked with the gold surface.[4,15,16] As shown in Figure 2A, the CD-SH layers do not cause the elimination of the couple due to the oxidation of Fc-OH (dashed line). On the other hand, ferricyanide is sterically incapable of complexing with β-CD, since ferricyanide is larger than the cavity of β-CD and so it cannot be included;[15,17] consequently the access of ferricyanide to the gold electrode is efficiently inhibited by the CD-SH layers and causes a complete inhibition of electron transfer in the reduction peak current (Figure 2B, dashed line).

The SECM technique also can be employed to study the electrochemical performance of modified electrodes; in this case the bare and CD-SH-modified electrodes are used as the SECM substrate. A mediator such as ferricyanide or Fc-OH is added to the solution, and the tip electrode is operated at a potential where the diffusion-controlled reduction, for ferricyanide, or oxidation, for Fc-OH, causes a steady-state current at the tip electrode in solution.

Figure 3 shows the approach curves from both Fc-OH and ferricyanide. To obtain a better comparison between experiments, the results are shown as a function of normalized tip current. Approach experiments carried out at a bare Au electrode (square symbols) reveal a positive feedback between the Pt tip and the Au electrode in concordance with the theory of SECM approach curves,[7,18] wherein the tip is brought close to a conductive surface, such as a bare Au electrode, and the mediator generated at the tip can undergo electron transfer and be restored to its original oxidation state. This regeneration of the mediator in the gap between the tip and substrate causes the current to increase, producing a positive feedback. According to this condition, the electron transfer at the substrate is diffusion limited, that is, $k° → ∞$.

On the other hand, in the approach curves carried out at a CD-SH-modified electrode (triangle symbols) there are two different behaviors. For Fc-OH, there is a positive feedback (Figure 3A), in a similar way as for the bare electrode, which implies that Fc-OH shows similar diffusion control processes at both electrodes, bare and modified. On the other hand, for ferricyanide, there is a negative feedback (Figure 3B), implying that the substrate (the CD-SH-modified electrode) acts as an insulating surface for ferricyanide, blocking diffusion of species from the tip, showing a decrease of the steady-state current. At this condition, the electron transfer on the substrate does not exist, that is, $k° → 0$.

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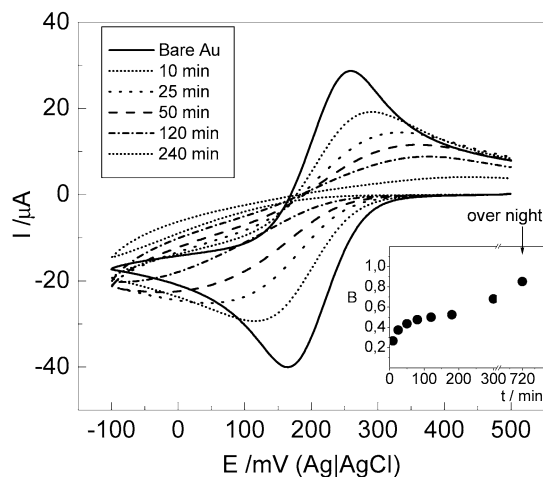
Figure 4. Cyclic voltammograms of 0.01 M ferricyanide in 0.1 M phosphate buffer (pH 7.0) at a gold electrode modified at different times with CD-SH. Sweep rate = 0.1 V s^{-1}. Inset: calculated hindrance B (eq 1) versus adsorption time. An overnight modification was considered as 12 h of modification (720 min).
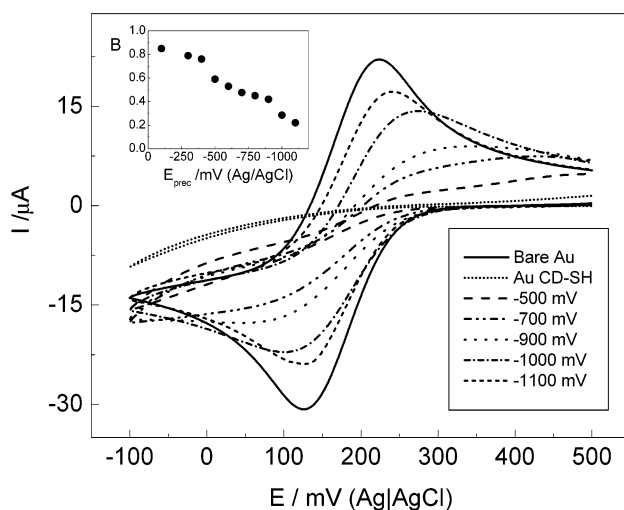
Consequently, these SECM results agree very well with those obtained by cyclic voltammetry, supporting that CD-SH forms an insulating film over the Au electrode for ferricyanide and a conductive surface for Fc-OH.

3.2. Surface Coverage and Reductive Desorption of CD-SH from the Gold Electrode. Taking advantage that electron transfer of ferricyanide is highly sensitive to the coverage of the gold surface, we select this reaction as a probe to study the adsorption and desorption of CD-SH at the gold electrode.

Figure 4 shows cyclic voltammograms of ferricyanide at the gold electrode modified with CD-SH after different adsorption times. The modified gold electrode was rinsed exhaustively before each voltammogram to remove physisorbed molecules. From this figure, it is clear that the coverage of the gold electrode increased with the adsorption time. The cyclic voltammograms of ferricyanide show two main changes as a consequence of the surface modification: first, a current decrease, and second, a loss of reversibility of the couple. Probably, these changes reveal that both the CD-SH-modified electrode area available for the redox processes and the heterogeneous electron transfer rate are smaller. According to the method previously described by Weisser et al.,[19] we calculated the hindrance (B) of the electrode using the following equation:

$$B = 1 - [i_p^f(CD)/i_p^f(Au)] \qquad (1)$$

where $i_p^f(CD)$ and $i_p^f(Au)$ are the forward (reduction of ferricyanide) peak currents measured at both the modified and the bare electrodes, respectively. Then, B is a qualitative parameter for the layer density. The inset of Figure 4 shows the dependence of hindrance B with the adsorption time, revealing a gradual increase of the covering. After ~720 min, a value of $B = 0.87$ was reached, indicating practically a totally covered Au surface. This result differs a little with that obtained by Wiesser et al., who obtained a kinetic curve with a plateau around $B = 0.85$ between 6 and 10 min. This difference could be attributed to the different way of modification of the electrode wherein Weisser et al. obtained a self-assembled monolayer using a vacuum procedure and we obtained a

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Figure 5. Cyclic voltammograms of 0.01 M ferricyanide in 0.1 M phosphate buffer (pH 7.0) at a preconditioned modified CD-SH gold electrode at different negative potentials for 40 s. Sweep rate = 0.1 V s^{-1}. Inset: calculated hindrance B (eq 1) versus preconditioning potential.

film of CD-SH working at room conditions of temperature and pressure. However, another important point of difference is that our cyclodextrin covering contained mainly CD pentathiolated and Weisser's covering was monothiolated.

The CD-SH layer formed on the gold surface was chemically stable, but there are two ways of removal, that is, mechanical polishing and electrochemical pretreatment. The covalent bond formed between Au and the −SH group is electrochemically reducible[12,20] according to

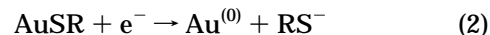$$AuSR + e^- \rightarrow Au^{(0)} + RS^- \qquad (2)$$

Figure 5 shows the cyclic voltammograms of ferricyanide at the CD-SH-modified gold electrode after different preconditioning potentials. We can observe that when the applied potential is more negative, the CD-SH suffers more desorption from the Au surface, as can be ascribed from the increased reduction of ferricyanide. The inset of Figure 5 shows the dependence of hindrance B with the applied potential, revealing a gradual decrease of the covering when the preconditioning potentials were more negative, reaching $B = 0.22$, revealing a practically uncovered Au surface.

Furthermore, we also have used SECM to electrochemically visualize the surface of gold when this surface is partially modified. Four approach curves done with the tip positioned at four different zones on the $x-y$ surface of the partially modified electrode ($B = 0.35$) are shown in Figure 6. On the other hand, the inset in Figure 6 reveals cyclic voltammograms of ferricyanide obtained with the partially modified electrode ($B = 0.35$) (dotted line) and at the bare electrode (solid line). These results show very well the difference of the microscopic approach obtained by SECM experiments when compared with the macroscopic approach obtained by CV experiments. In the case of the CV measurements, we obtained only one CV curve for the partially modified electrode, but in the case of the SECM experiments we obtained four different approach curves with different feedback currents depending on the tip position on the partially modified electrode surface. Furthermore, we can appreciate that the approach curves

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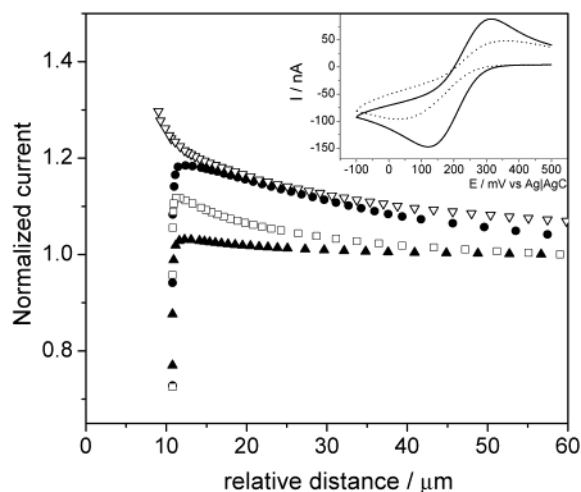
Figure 6. Normalized tip current vs relative distance curves for the tip approaching the different zones of a CD-SH partially modified gold electrode; 0.01 M ferricyanide in 0.1 M phosphate buffer (pH 7.0). $E_{tip} = -100$ mV, and $E_s = 500$ mV. Inset: cyclic voltammograms of 0.01 M ferricyanide in 0.1 M phosphate buffer (pH 7.0) at bare (solid line) and partially modified CD-SH ($B = 0.35$, dotted line) gold electrodes. Sweep rate = 0.1 V s^{-1}.

show different behaviors ranging from a totally positive (open triangle) to a totally negative (solid triangle) feedback current, but in the intermediate states the situation is rather different showing a tip current increase and then decay originating a peak-shaped response. Bard and co-workers described very well that phenomenon,[2,6,18] concluding that the peak-shaped response is a result of the intermixing of insulator and conductor behavior; then it is possible to conclude that in the present case the electron-transfer rate at the conductor surface ($k_{b,s}$) is less than the diffusion limit (i.e., is a nonreversible surface) and therefore the feedback current is a function of several parameters, the standard rate constant, the tip shape, the tip-to-substrate distance, the substrate potential, and so forth.

According to the Butler–Volmer equation,[21] the rate constant for the one-electron oxidation of ferrocyanide, $k_{b,s}$, at the modified gold electrode is described by

$$k_{b,s} = k° \exp[\alpha F/RT(E_s - E°')] \qquad (3)$$

where $k°$ is the standard rate constant, $E°'$ is the formal potential, α is the transfer coefficient, F is the Faraday constant, R is the gas constant, and T is the temperature. As in these experiments we keep constant both the tip (-100 mV) and the substrate (500 mV) potentials, the overpotential is constant and consequently the E_s is not the rate-determining factor for the approach curves, then a reasonable explanation to obtain intermediate approach curves between negative feedback ($k°$ and $k_{b,s} \rightarrow 0$) and positive feedback ($k°$ and $k_{b,s} \rightarrow \infty$) is that the ferricyanide was regenerated by the modified Au electrode at a measurable rate, that is, $0 < k° < \infty$; then the surface of the modified electrode must be conductive in some parts of its surface.

This fact is also in accord with the observed results in the cyclic voltammograms displayed in the inset of Figure 6, wherein a notorious loss of reversibility was induced when the electrode was partially modified. However, this quasireversible cyclic voltammogram is a macroscopic measurement obtained from the overall electrode surface,

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meaning an average of the many independent $k°$ that are distributed through the partially modified electrode. An adequate microscopic discrimination can be evaluated by SECM.

3.3. Imaging of the CD-SH Gold Modified Electrode with SECM. Using the same medium and the same electrochemical reactions, we employed the SECM peculiarities to electrochemically visualize the bare and modified surface Au electrodes. The principle of these experiments was the same as that in the approach experiments. As the microelectrode tip was brought near the surface, the SECM image is obtained by two-dimensional mapping of reduction, for ferricyanide, and/or oxidation, for Fc-OH, current versus the tip position, wherein the tip had a determined applied potential and the substrate had the opposite potential.

Figure 7 shows the SECM images obtained from bare and partially and totally modified gold electrodes using ferricyanide as the electrochemical mediator. All the experiments were done at the same distance between tip and substrate ($d = 10$ μm). The results are shown with reference to the normalized tip current (I_{norm}), which permits a better comparison between different experiments. Also, the corresponding cyclic voltammograms for each electrode condition are displayed (Figure 7A). When the electrode is nonmodified (Figure 7B), it is possible to observe that the current is about 1.5 times greater than the diffusion current, and the current is homogeneous in the entire explored surface, that is, the topography is regular. On the other hand, when the electrode is totally modified (Figure 7C), also a homogeneous current is observed (a regular topography), but this current corresponds only to the tip current generation ($I_{norm} = 1$), which represents a nonexistent feedback with the substrate. In the intermediate situation of covering (Figure 7D–F), we can observe that the normalized current reaches an intermediate value when compared with the bare and totally modified electrode images. Moreover the surface is not homogeneous; there are zones of the electrode surface wherein higher currents are produced. This fact gives a reliable explanation for the above results obtained with the feedback experiments at the partially modified electrode, wherein the approach curves evidenced the differences in conductance. Another conclusion that we can obtain from the SECM images is the fact that when the electrode is less modified, $B = 0.22$, Figure 7D, the surface topography is more irregular and when the modification increases, $B = 0.35$ and $B = 0.4$, Figure 7E and Figure 7F, respectively, the topography becomes more regular and the normalized current is lower.

On the other hand, we have also used Fc-OH as the mediator between the tip and substrate, to obtain surface topography of the bare and totally modified electrodes. In this case, as Fc-OH forms an inclusion complex with the CD cavity, we found that there was no difference between the two experiments, and the normalized current was always higher than 1, reaching values near 1.4 and 1.5 (data not shown).

3.4. Kinetic Evaluation of the ($[Fe(CN)_6]^{4-}$) Oxidation, $k_{b,s}$, at the Modified Gold Electrode. According to the Butler–Volmer equation (eq 3), the heterogeneous rate constant of ferrocyanide oxidation, $k_{b,s}$, at the CD-modified gold electrode can be increased by modifying the substrate potential E_s, that is, using a more positive potential in this case, which will produce higher overpotential and finally higher $k_{b,s}$. This fact has been also
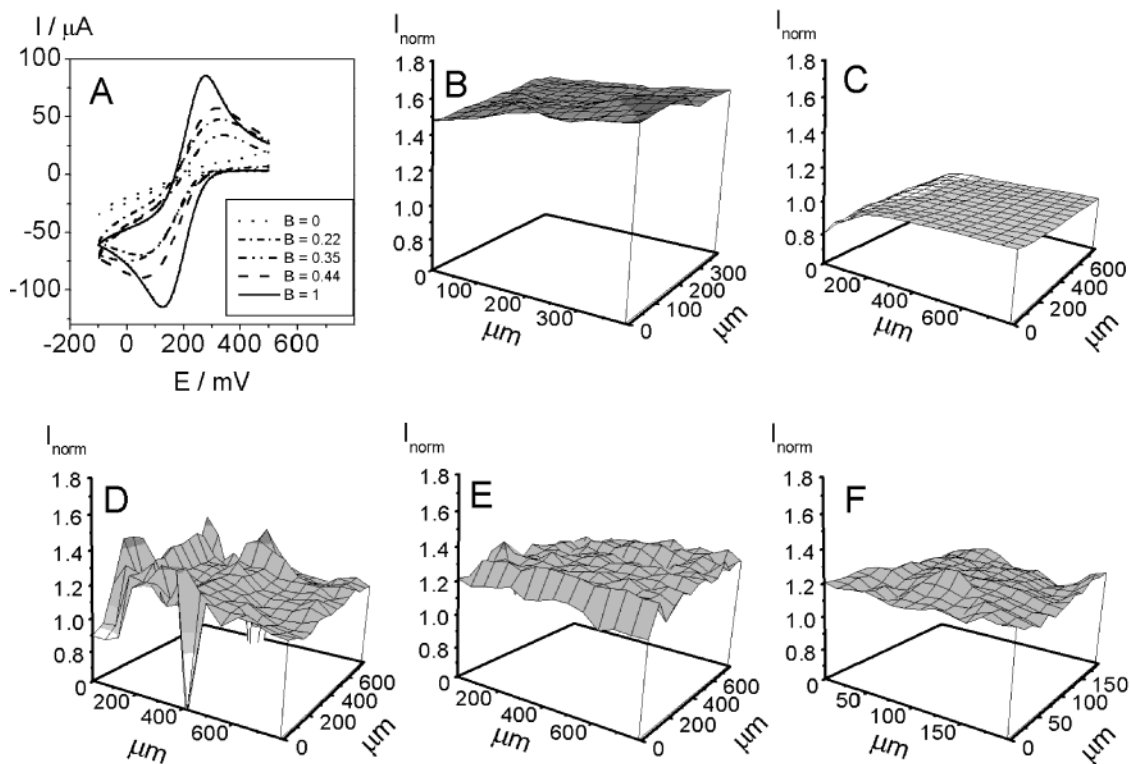
Figure 7. (A) Cyclic voltammograms of 0.01 M ferricyanide in 0.1 M phosphate buffer (pH 7.0) at a gold electrode modified at different times with CD-SH. Sweep rate = 0.1 V s^{-1}. (B–F) SECM surface-plot images of bare (B), totally modified ($B = 1$) (C), and partially modified (D–F) CD-SH electrodes: $B = 0.22$ (image D), $B = 0.35$ (image E), and $B = 0.44$ (image F). The images were made with an amperometric Pt tip ($a = 5$ μm, rate = 100 μm s^{-1}) at about 10 μm of distance (d); $E_{tip} = -100$ mV, and $E_s = 500$ mV. 0.01 M ferricyanide in 0.1 M phosphate buffer (pH 7.0) was used as a mediator.
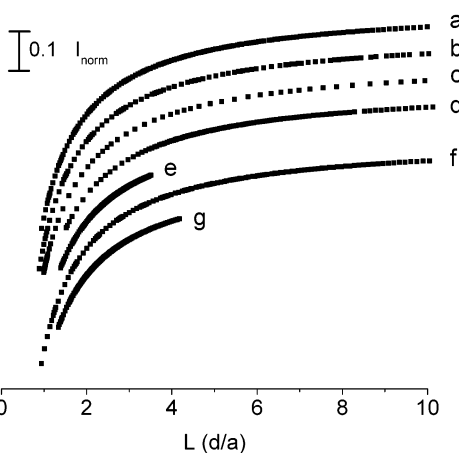
Figure 8. Normalized tip current–distance curves for the tip approaching the totally CD-SH modified gold electrode at different substrate potentials: 400 mV (a), 500 mV (b), 600 mV (c), 700 mV (d), 800 mV (e), 900 mV (f), and 1000 mV (g); 0.01 M ferricyanide in 0.1 M phosphate buffer (pH 7.0). $E_{tip} = -100$ mV for ferricyanide reduction. Pt tip radius = 5 μm.

observed with other modified electrodes with ferricyanide, performing a total reversion of the negative to positive feedback.[22]

Approach curves obtained at different substrate applied potentials, from 500 to 1000 mV, are displayed in Figure 8. There are no changes in the shape of the curves,

remaining in a negative feedback form, implying that although the potential is increased substantially and so therefore is the overpotential value, the modified electrode is unable to produce electron transfer and remain insulating for ferricyanide.

4. Conclusions

We successfully applied cyclic voltammetry and SECM experiments to surface studies. The β-CD-SH-modified gold electrode was characterized and can be used as a selective, conductive or an insulating substrate for CV and SECM experiments, depending on the host–guest interaction that involves the cyclodextrin, that is, if the mediator molecule can enter or not into the β-cyclodextrin cavity, forming or not an inclusion complex with the β-CD.

This work provides a better understanding of the intermediate stages of the electrode modification with β-CD derivate. Moreover, it is a very clear example wherein electrochemists can easily visualize the difference between doing electrochemistry at macroscopic (CV) and microscopic levels (SECM).

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