

# Interfacial Molecular Recognition. Binding of Ferrocenecarboxylate to $\beta$ -Aminocyclodextrin Hosts Electrostatically Immobilized on a Thioctic Acid Monolayer<sup>†</sup>

Yun Wang and Angel E. Kaifer\*

Chemistry Department, University of Miami, Coral Gables, Florida 33124-0431

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The interfacial binding properties of electrodes prepared by the electrostatic immobilization of an adlayer of positively charged  $\beta$ -aminocyclodextrin ( $\beta$ -ACD) hosts on a self-assembled monolayer of thioctic acid on gold were investigated using Osteryoung square wave voltammetry. The results indicate that the water-soluble ferrocenecarboxylate ( $\text{Fc-COO}^-$ ) guest was effectively bound by the interfacial  $\beta$ -ACD hosts, with binding constants in the range  $(2-5) \times 10^4 \text{ M}^{-1}$ . The measured binding constants were found to increase slightly with the surface density of immobilized hosts.

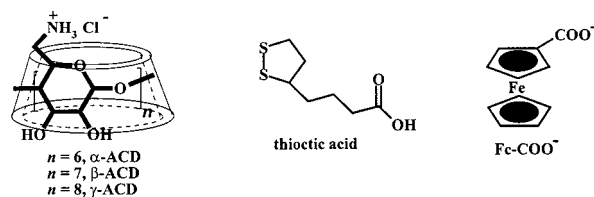
## Introduction

Our group<sup>1</sup> and others<sup>2</sup> have described the incorporation of host molecules into self-assembled monolayer (SAM) structures. The fundamental idea behind this work is to express the binding selectivity properties of the host in the electrochemical reactions taking place at the electrode–solution interface. Obvious applications to the development of new voltammetric sensors might result from these research efforts.<sup>3</sup> The cyclodextrins<sup>4</sup> (CDs) are promising hosts for this purpose because they are widely available, their binding properties in homogeneous solution have been extensively investigated, and their functionalization chemistry has also been the subject of considerable synthetic work.<sup>5</sup> In 1995, our group reported the chemisorption of a thiolated  $\beta$ -CD derivative on gold surfaces and the selective binding properties of the resulting monolayers for the guest ferrocene.<sup>1a</sup> More recently, while searching for alternative procedures to prepare organized interfacial assemblies of CD hosts, we have investigated the aggregation of amphiphilic, positively charged cyclodextrins on gold surfaces derivatized with carboxylate-terminated SAMs.<sup>6</sup> In this case, the formation of thick multilayer aggregates was detected from voltammetric and QCM measurements. Although multilayer aggregates are interesting in their own right, their structure and thickness may hinder the interfacial molecular recognition processes, which constitute the primary reason to justify this work. Therefore, we decided to continue looking for appropriate methods for the preparation of interfacial monolayer assemblies containing CD hosts.

Thioctic acid SAMs have been previously reported by other groups.<sup>7</sup> They offer an alternative to monolayers prepared from  $\omega$ -mercaptocarboxylic acids. The cyclic disulfide functionality of thioctic acid affords two anchoring points to the gold surface per chemisorbed molecule, a fact that may lead to more stable SAMs. Here, we report the results of an investigation on host–guest phenomena with an interfacial system designed around the electrostatic immobilization of the positively charged  $\beta$ -amino-CD host ( $\beta$ -ACD) on negatively charged SAMs, which, in turn, are prepared by the chemisorption of thioctic acid on gold surfaces.

<sup>†</sup> Dedicated to Professor Allen J. Bard on the occasion of his 65th birthday.

## STRUCTURES



## Experimental Section

**Materials.** 6-Deoxy-6-amino- $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrins were synthesized according to published procedures.<sup>8</sup> 6,8-Dithioctic acid (TOA) was purchased from Lancaster Synthesis and used without further purification. Boric acid, ferrocenecarboxylic acid, potassium ferricyanide, and 1-adamantanecarboxylic acid were purchased from Aldrich and used as received.  $\text{Ru}(\text{NH}_3)_6\text{Cl}_3$  was obtained from Strem Chemicals and used without further purification. All other chemicals were of the best quality commercially available. Gold wire (99.999%, 0.5 mm diameter) was purchased from Johnson Matthey. Deionized water was further purified by passage through a pressurized, four-cartridge Barnstead Nanopure system to a final resistivity of  $18 \text{ M}\Omega\cdot\text{cm}$  or higher.

**Equipment.** All the electrochemical experiments were performed with a BAS 100 B/W workstation (Bioanalytical Systems, West Lafayette, IN). A gold bead working electrode, Pt counter electrode, and  $\text{Ag}/\text{AgCl}$  reference electrode were fitted in a 10 mL, single-compartment electrochemical cell. The cell resistance was compensated by the BAS 100 B/W instrument.

**Procedures.** The gold bead working electrodes were made by annealing the tip of a gold wire in a gas–oxygen flame. The roughness factors of these electrodes were measured to be in the range of 1.1–1.2.<sup>9</sup> The geometric areas of the gold electrodes were calculated from the slopes of the linear plots of cathodic peak current versus the square root of the scan rate obtained for the diffusion-controlled reduction of  $\text{Ru}(\text{NH}_3)_6^{3+}$ . The diffusion coefficient of  $\text{Ru}(\text{NH}_3)_6^{3+}$  was previously determined to be  $7.5 \times 10^{-6} \text{ cm}^2/\text{s}$  (at  $25^\circ\text{C}$  in 0.1 M NaCl).<sup>10</sup>

Typical values for the geometric area of the electrode varied from 0.010 to 0.015 cm<sup>2</sup>.

After its preparation, the gold bead electrode was rinsed with a large amount of purified water and immersed in a 1 mM solution of dithioctic acid in 2:1 (v/v) ethanol/H<sub>2</sub>O for at least 24 h. The monolayer-covered gold electrode (Au/TOA) was rinsed with 2:1 ethanol/H<sub>2</sub>O and dried in air before use. Commonly, the formation of the TOA monolayer on the electrode was detected by the complete blocking of the cathodic wave of 1 mM Fe(CN)<sub>6</sub><sup>3-</sup> in pH 8 boric acid buffer solution also containing 0.1 M NaCl. The pH 8 boric acid buffer solution was made by adjusting the pH of 5 mM boric acid aqueous solution with 0.1 M NaOH. Electrodes on which the Fe(CN)<sub>6</sub><sup>3-</sup> voltammetric response was incompletely blocked were discarded.

$\beta$ -Aminocyclodextrin ( $\beta$ -ACD) was electrostatically adsorbed to electrodes modified with TOA monolayers by soaking them for 10 min in a pH 8 boric acid buffer solution containing variable concentrations of  $\beta$ -ACD (0.5–5.0 mM). The interfacial complexation phenomena between the ferrocenecarboxylate (Fc-COO<sup>-</sup>) guest and the interfacial  $\beta$ -ACD host were studied by Osteryoung square wave voltammetry (OSWV). The Au/TOA/ $\beta$ -ACD-modified electrode was removed from the  $\beta$ -aminocyclodextrin solution and rinsed with pure water then immersed in a pH 8 boric acid buffer solution containing different concentrations of Fc-COO<sup>-</sup> (30  $\mu$ M–0.3 mM). The first OSWV was recorded exactly 30 s after transfer of the electrode from the  $\beta$ -ACD solution to the Fc-COO<sup>-</sup> solution. The OSWV was recorded again after 10 min, when the OSWV response had reached a steady state. The difference OSWV, which reflects only the oxidation of surface-confined Fc-COO<sup>-</sup> (vide infra), was simulated following a method reported by Bowden and co-workers.<sup>11</sup> Fitting of the simulation to the difference OSWV was used to determine the surface coverage of Fc-COO<sup>-</sup> as a function of the concentration of Fc-COO<sup>-</sup> in the bulk solution.

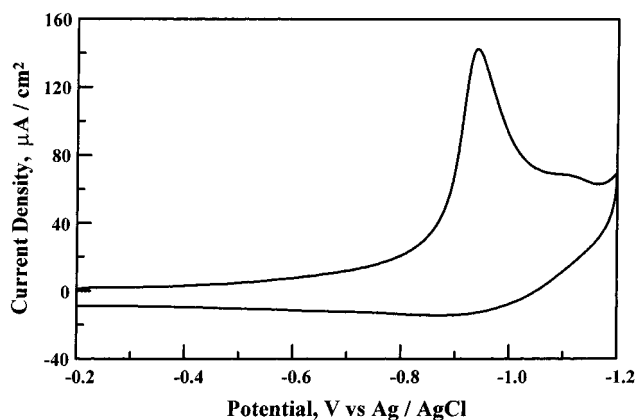
## Results and Discussion

**Thioctic Acid Monolayers.** The formation and characterization of stable self-assembled thioctic acid (TOA) monolayers has been reported.<sup>7</sup> On the basis of these reports, we prepared monolayers by exposing clean gold bead electrodes to a 1.0 mM TOA solution in ethanol/water (2:1, v/v) for 24 h. The modified gold electrodes exhibited a cyclic voltammetric response in 0.5 M KOH characteristic of monolayer desorption, with an irreversible cathodic wave at ca. -1.0 V, which corresponds to the reductive desorption of the surface-attached thiolates. This cathodic process is represented by the following equation:

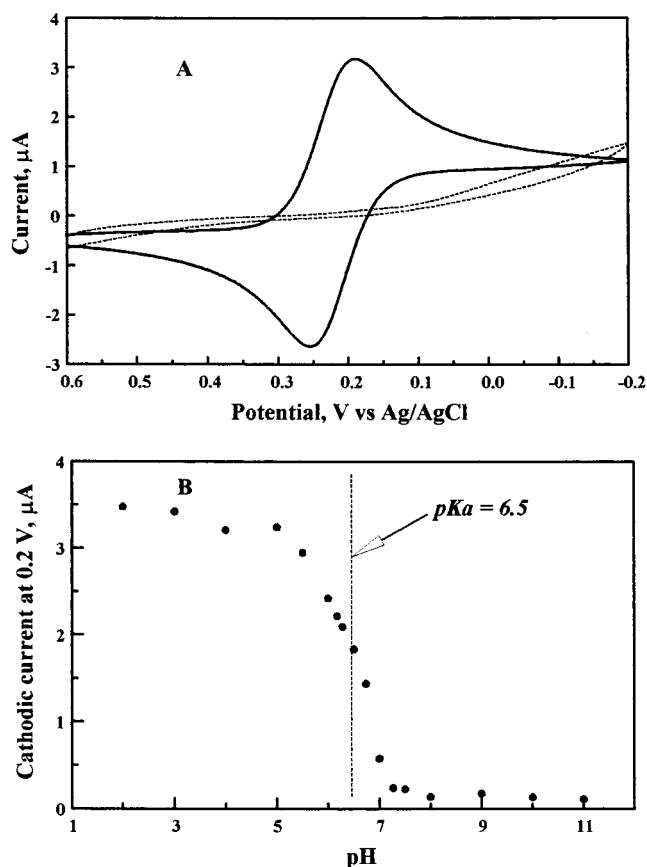


A typical voltammogram is shown in Figure 1. Integration of the current under the cathodic wave provides an estimated surface coverage for the TOA molecules of  $(3.1 \pm 0.5) \times 10^{-10}$  mol/cm<sup>2</sup>. However, the reliability of reductive desorption measurements to determine thiolate surface coverages on gold has been questioned.<sup>12</sup>

According to previous reports,<sup>7</sup> TOA monolayers should be neutral at low pH as the carboxylic acid groups are protonated and develop negative charges in neutral and basic solutions due to the ionization of the terminal COOH groups. Fe(CN)<sub>6</sub><sup>3-</sup>, which is negatively charged and undergoes fast one-electron reduction, was chosen as a probe to detect the degree of



**Figure 1.** Cyclic voltammogram of Au/TOA-modified electrode in 0.5 M KOH; scan rate = 0.1 V/s.



**Figure 2.** (A) Cyclic voltammetric response of a Au/TOA-modified electrode of 1.0 mM Fe(CN)<sub>6</sub><sup>3-</sup>, 0.1 M NaCl at pH 2 (solid line) and at pH 8 (dotted line); scan rate = 0.1 V/s. (B) The pH Dependence of the cathodic current measured at 0.2 V on a Au/TOA-modified electrode immersed in 1.0 mM Fe(CN)<sub>6</sub><sup>3-</sup> solution also containing 0.1 M NaCl. The pH was adjusted by addition of HCl or NaOH.

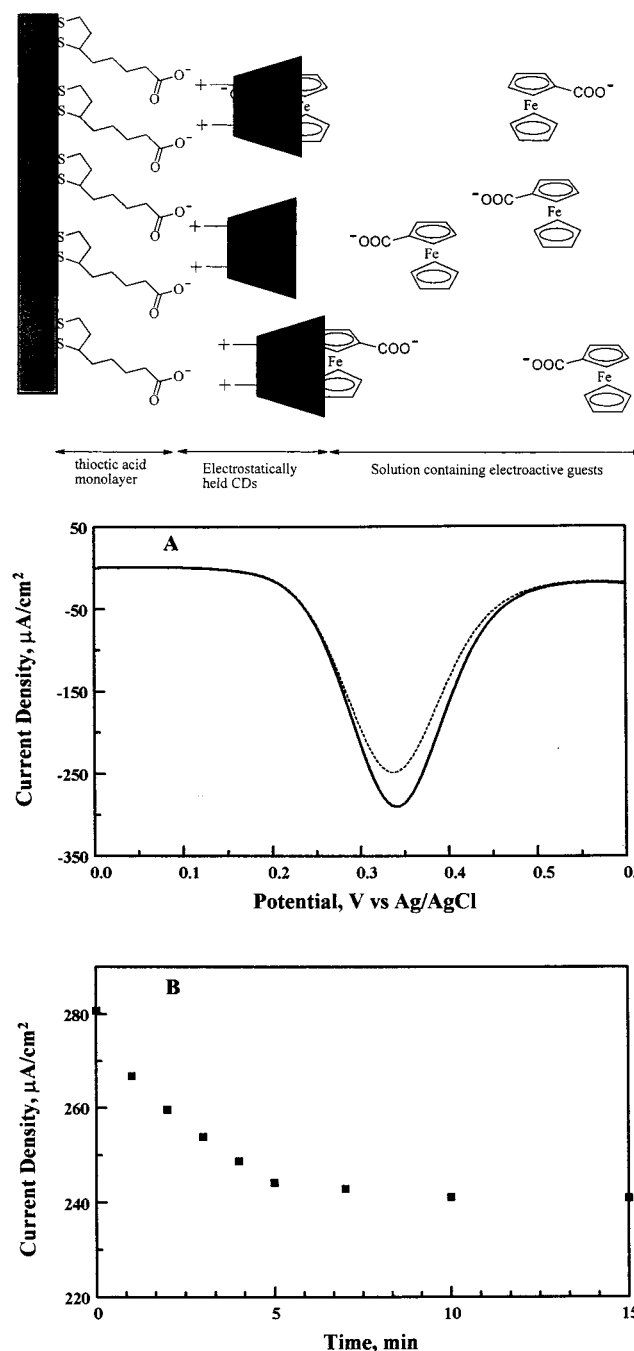
protonation of TOA monolayers. As anticipated, the observed CV behavior of Fe(CN)<sub>6</sub><sup>3-</sup> on Au/TOA-modified electrodes strongly depends on the pH of the solution. At pH 2, the TOA monolayer is fully protonated and a reversible cyclic voltammetric response was observed [Figure 2A (solid line),  $E_{p,c} = 0.20$  V,  $E_{p,a} = 0.26$  V,  $i_{p,c} = 3.49$   $\mu$ A], while the response was largely blocked in basic solution (pH > 7) upon deprotonation of the TOA molecules due to the repulsion between the Fe(CN)<sub>6</sub><sup>3-</sup> ions and the negatively charged monolayer surface [Figure 2A (dotted line)]. The cathodic current at 0.2 V was monitored as the pH of the solution was titrated from 2 to 11

(Figure 2B). This plot clearly exhibits a point of inflection at a pH value of approximately 6.5, which represents an estimated value for the  $pK_a$  of the TOA monolayer COOH groups. This value is about 1.5 units higher than that measured for thioctic acid in bulk aqueous solution ( $pK_a = 5$ ). This finding is in excellent agreement with other reports,<sup>13</sup> which have shown that the  $pK_a$  of monolayer COOH groups is substantially higher than the corresponding value in homogeneous solution.

**Electrostatic Immobilization of  $\beta$ -Aminocyclodextrin ( $\beta$ -ACD) onto TOA Monolayers.** Carboxylate-terminated SAMs can provide negatively charged surfaces if the pH of the contacting solution is sufficiently high. Several reports have shown that these SAMs can serve as effective templates for the irreversible adsorption of molecules with positive charges via electrostatic interactions.<sup>13a,14</sup> In this work, we investigated the immobilization of  $\beta$ -ACD receptor molecules onto TOA monolayers using the favorable interactions between the crown of positive charges ( $NH_3^+$ ) on the  $\beta$ -ACD hosts and the negative charges on the monolayer. Previous studies suggested that the optimization of solution conditions is crucial for efficient electrostatic adsorption.<sup>15</sup> In our experiments, the selected medium for electrostatic adsorption was a solution containing variable concentrations of  $\beta$ -ACD and 5 mM boric acid, which was adjusted to pH 8 with 0.1 M NaOH. This medium was selected due to four main reasons. (1) According to the  $pK_a$  values of TOA monolayers and  $\beta$ -ACD,<sup>16</sup> essentially all the COOH groups of TOA are deprotonated and about half of the  $-NH_2$  groups of  $\beta$ -ACD are still protonated at pH 8. (2) Boric acid buffer was used instead of phosphate buffer because  $\beta$ -ACD binds phosphate ion selectively over monovalent anions<sup>17</sup> and at pH 8 only a monovalent anion ( $H_2BO_3^-$ ) is present in the solution ( $pK_{a1} = 9.14$  for boric acid). (3) The low ionic strength of the solution (5 mM boric acid) enhances the electrostatic interactions between the TOA monolayer and the  $\beta$ -ACD hosts. (4) Different concentrations of  $\beta$ -ACD were used because the surface density of adsorbed  $\beta$ -ACD hosts can be controlled by changing the concentration of  $\beta$ -ACD in the bulk solution. The last point is particularly interesting, since it will allow us to study the effect of the surface density of  $\beta$ -ACD on the interfacial complexation reactions.

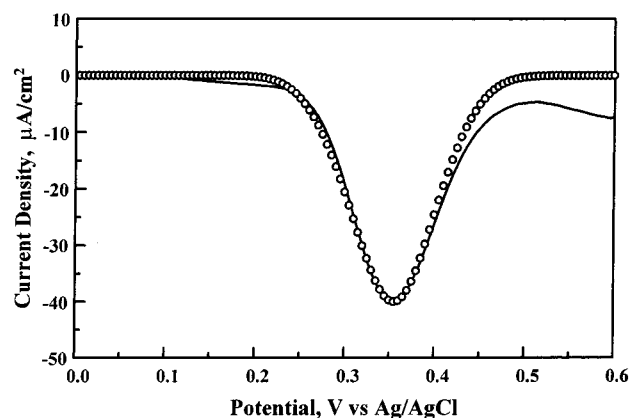
**Interfacial Binding Studies.** To use electrochemical measurements to investigate the binding properties of the interfacial  $\beta$ -ACD hosts, electrodes modified with TOA monolayers were further modified as described above by immersion in solutions containing variable concentrations of  $\beta$ -ACD (Au/TOA/ $\beta$ -ACD) and finally introduced into pH 8 boric acid buffer solutions containing ferrocenecarboxylate ( $Fe-COO^-$ ), a well-known electroactive guest for the  $\beta$ -ACD host. Compared with ferrocene and its other derivatives (e.g., ferrocenemethanol),  $Fe-COO^-$  was chosen because of its higher water solubility and its higher binding constant with  $\beta$ -ACD,<sup>17</sup> which is enhanced by the favorable interaction between its carboxylate group and the positive charges on  $\beta$ -ACD. An idealized representation of these interfacial binding experiments is shown in Scheme 1. OSWV was used to investigate the binding interactions in this monolayer system due to its convenient analytical sensitivity. The OSWV response for the oxidation of  $Fe-COO^-$  at a Au/TOA/ $\beta$ -ACD-modified electrode in  $\beta$ -ACD-free solution decreased with time and reached a steady state after 10 min (Figure 3). The gradual current decrease follows a time evolution that is identical to that observed for the voltammetric reduction of  $Ru(NH_3)_6^{3+}$  on the same electrode surface.<sup>18</sup> It is reasonable to correlate both phenomena with the slow redissolution of the  $\beta$ -ACD hosts from the electrode surface. Therefore, the higher

### SCHEME 1: Interfacial Molecular Architecture



**Figure 3.** (A) OSWV response of a Au/TOA/ $\beta$ -ACD-modified electrode of a 0.3 mM  $Fe-COO^-$  in pH 8 boric acid buffer solution recorded 30 s after transfer (solid line) and 10 min after transfer (dotted line) of the electrode into the  $Fe-COO^-$  solution. (B) Time dependence of the observed peak current in the experiment of Figure 3A; step potential = 5 mV.; square wave amplitude = 25 mV.; frequency = 100 Hz.

current response observed shortly after the immersion of the Au/TOA/ $\beta$ -ACD in the solution containing the  $Fe-COO^-$  guest indicates that  $Fe-COO^-$  is preconcentrated at the electrode surface due to the presence of interfacial  $\beta$ -ACD hosts. The first OSWV, which was recorded 30 s after transfer of the Au/TOA/ $\beta$ -ACD electrode into the  $Fe-COO^-$  solution, results from the oxidation of  $Fe-COO^-$  confined at the electrode surface by binding to the interfacial  $\beta$ -ACD as well as from the oxidation of freely diffusing  $Fe-COO^-$  molecules. As the electrostatically held  $\beta$ -ACD hosts gradually desorb into the solution, the current

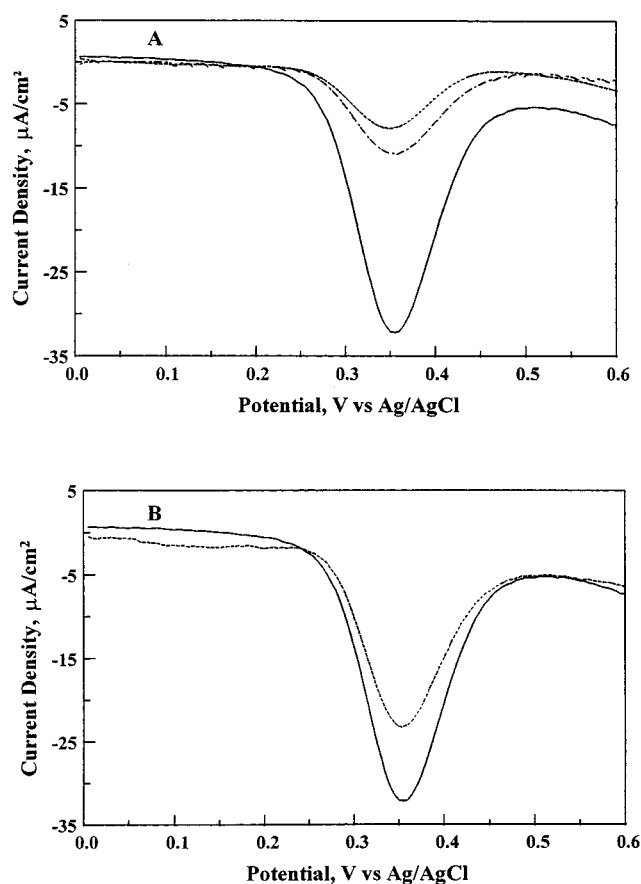


**Figure 4.** Difference OSWV (solid line) obtained by subtraction of the two OSWV's in Figure 3A. The circles were calculated by digital simulation.

levels observed for the oxidation of  $\text{Fc-COO}^-$  decrease because less and less guest can be bound at the interface and, thus, preconcentrated at the electrode surface. After 10 min, the current reaches a steady-state level, effectively indicating the end of the desorption of the interfacial hosts. This steady-state OSWV response is essentially identical to the OSWV response obtained at the Au/TOA electrode before modification with  $\beta$ -ACD and results solely from the electrochemical oxidation of freely diffusing  $\text{Fc-COO}^-$ . Therefore, the difference OSWV, which is obtained by subtracting the steady-state voltammogram from the first recorded OSWV, reflects only the oxidation of surface-confined  $\text{Fc-COO}^-$ . The OSWV response of surface-confined  $\text{Fc-COO}^-$ , i.e., the difference OSWV discussed above, can be easily simulated by the method reported by Bowden and co-workers.<sup>11</sup> Thus, the surface coverage of  $\text{Fc-COO}^-$  can be determined from the optimization of the fitting between simulations and the difference OSWV (Figure 4) obtained from the experimental results.

Several control experiments were carried out in order to corroborate the origin of the difference OSWV response. First, a bare gold electrode was used instead of the Au/TOA-modified electrode for the electrostatic adsorption of  $\beta$ -ACD. No difference OSWV could be detected in this case when this electrode was immersed in solutions containing  $\text{Fc-COO}^-$ , that is, a steady-state OSWV response was reached immediately. This result confirms that the driving force for the adsorption of the  $\beta$ -ACD hosts onto the electrode surface is their electrostatic interaction with the negatively charged surface of the electrode covered with the TOA monolayer. In the absence of the TOA monolayer, no interfacial adsorption of  $\beta$ -ACD was detected and no surface excess of  $\text{Fc-COO}^-$  could be measured.

In a different series of control experiments,  $\alpha$ - and  $\gamma$ -ACD-modified electrodes were prepared by immersing the Au/TOA-modified electrode in a solution containing  $\alpha$ - or  $\gamma$ -ACD, instead of  $\beta$ -ACD. With either one of these electrodes, the difference OSWVs measured in solutions containing  $\text{Fc-COO}^-$  were much smaller than that observed on the  $\beta$ -ACD-modified electrode (Figure 5A). The relative size of the difference OSWV responses obtained with  $\alpha$ -,  $\beta$ -, and  $\gamma$ -ACD correlate very well with the known binding constants of simple ferrocene derivatives with  $\alpha$ -,  $\beta$ -, and  $\gamma$ -CD.<sup>19</sup> This finding constitutes a strong indication that the interfacial accumulation of  $\text{Fc-COO}^-$  results from its binding to the CD receptors electrostatically held on the TOA monolayer surface. Finally, the  $\beta$ -ACD-modified electrode was immersed in a solution containing not only  $\text{Fc-COO}^-$  but also 1-adamantane carboxylic acid ( $\text{Ad-COO}^-$ ), which is another excellent but electroinactive guest for the

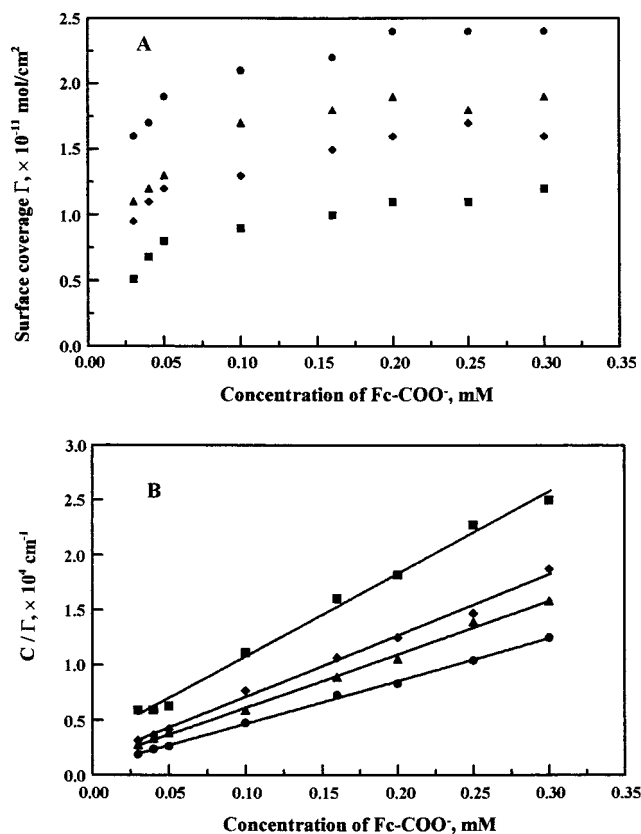


**Figure 5.** (A) Difference OSWVs measured in pH 8 boric acid buffer containing  $50 \mu\text{M}$   $\text{Fc-COO}^-$  on electrodes modified with  $\alpha$ -ACD (dotted line),  $\beta$ -ACD (solid line), and  $\gamma$ -ACD (- · - ·) adlayers, prepared by immersing Au/TOA electrodes for 10 min in pH 8 boric acid buffer solution containing 3.0 mM  $\alpha$ -,  $\beta$ -, or  $\gamma$ -ACD, respectively. (B) Difference OSWVs measured on a Au/TOA/ $\beta$ -ACD-modified electrode in pH 8 boric acid buffer containing  $50 \mu\text{M}$   $\text{Fc-COO}^-$  (solid line) and  $50 \mu\text{M}$   $\text{Fc-COO}^- + 50 \mu\text{M}$   $\text{Ad-COO}^-$  (dotted line).

$\beta$ -ACD host. The results of this competition experiment are clearly shown in Figure 5B. Addition of  $50 \mu\text{M}$   $\text{Ad-COO}^-$  to a solution containing  $50 \mu\text{M}$   $\text{Fc-COO}^-$  causes a substantial decrease in the difference OSWV associated with the oxidation of surface-confined  $\text{Fc-COO}^-$ . This finding indicates that  $\text{Ad-COO}^-$  competes effectively with  $\text{Fc-COO}^-$  molecules for the same set of  $\beta$ -ACD binding sites. The surface-confined  $\text{Ad-COO}^-$  ions are not detectable by OSWV, since they are not electroactive.

The experiments described so far clearly establish that the electrostatic adsorption of  $\beta$ -ACD on the surface of Au/TOA electrodes gives rise to a measurable surface excess of the electroactive guest ( $\text{Fc-COO}^-$ ), which results from its complexation by the electrostatically held  $\beta$ -ACD hosts. As the hosts are not irreversibly adsorbed on the surface of the TOA monolayer, they are gradually lost to the solution in a desorption process that takes place over a period of 10 min. Therefore, in these experiments, we monitor the interfacial  $\text{Fc-COO}^-$ - $\beta$ -ACD complexation phenomena while the  $\beta$ -ACD hosts undergo slow desorption from the electrochemical interface. We must address the question of whether the desorption of the hosts prevents us from obtaining any equilibrium data on the interfacial host-guest complexation phenomena. To answer this question, it is important to point out that the complexation reaction takes place at considerably faster rates than those for the observed host desorption process. The complexation between most substrates





**Figure 6.** (A) Measured  $\text{Fc-COO}^-$  surface coverages for several types of Au/TOA/ $\beta$ -ACD-modified electrodes as a function of the  $\text{Fc-COO}^-$  concentration in the contacting pH 8 boric acid buffer solution. The Au/TOA/ $\beta$ -ACD-modified electrodes were prepared by changing the concentration of  $\beta$ -ACD in the "loading" solution: 0.5 mM (■), 1.0 mM (◆), 3.0 mM (▲), 5.0 mM (●). (B)  $C/\Gamma$  vs  $C$  Langmuir plots for the data of Figure 6A.

and cyclodextrin hosts in homogeneous solution is known to be a kinetically fast process, with complex lifetimes on the order of milliseconds.<sup>4</sup> In our previous work with cyclodextrins and other receptors covalently attached to the electrode surface,<sup>1</sup> binding equilibrium with solution guests was always reached quickly enough so that kinetic effects were never observed in the time scale (seconds) of voltammetric experiments. Since desorption of the  $\beta$ -ACD hosts was observed to take place over a period of  $\sim 10$  min, we conclude that the interfacial hosts are in equilibrium (or very close to equilibrium) with the  $\text{Fc-COO}^-$  guests in the solution. To eliminate the influence of the slow desorption of the hosts in our data, we always measured the first OSWV response exactly 30 s after immersing the Au/TOA/ $\beta$ -ACD in the guest solution. This affords enough time for host–guest equilibration while minimizing the loss of desorbed host to the solution. The steady-state OSWV response (obtained more than 10 min after electrode transfer) was subsequently subtracted from the OSWV recorded initially (after 30 s) to yield the voltammetric response due to the surface-confined  $\text{Fc-COO}^-$  guests.

To investigate more quantitatively the complexation of  $\text{Fc-COO}^-$  by interfacial  $\beta$ -ACD hosts, we exposed Au/TOA/ $\beta$ -ACD-modified electrodes to solutions containing increasing  $\text{Fc-COO}^-$  concentrations. Using the already described experimental protocol, we found that the surface excess of  $\text{Fc-COO}^-$  markedly depends on its bulk solution concentration. Plots of the measured surface excess of the guest as a function of its bulk concentration exhibit shapes consistent with binding isotherms (Figure 6A), a finding which agrees with the proposed host–

**TABLE 1: Langmuir Parameters for the Adsorption/Complexation of  $\text{Fc-COO}^-$  on Au/TOA/ $\beta$ -ACD Electrodes<sup>a</sup>**

$[\beta\text{-ACD}]$ (mM) <sup>b</sup>	$\Gamma_{\text{max}}$ ( $\times 10^{-11}$ mol/cm <sup>2</sup> )	$K$ ( $\times 10^4$ M <sup>-1</sup> )
0.5	1.3	2.3
1.0	1.8	3.6
3.0	2.0	3.9
5.0	2.6	5.0

<sup>a</sup> Data obtained from OSWV measurements at 25 °C. <sup>b</sup> Concentration of  $\beta$ -ACD in the solution used to prepare the host adlayer.

guest complexation process between the  $\beta$ -ACD hosts and the  $\text{Fc-COO}^-$  guests. If we assume that each  $\beta$ -ACD binding site acts independently from the rest, the experimental data should follow the Langmuir adsorption isotherm, which can be conveniently expressed as

$$C\Gamma^{-1} = C(\Gamma_{\text{max}})^{-1} + (K\Gamma_{\text{max}})^{-1} \quad (2)$$

where  $C$  is the bulk concentration of guest,  $\Gamma$  is the experimental surface coverage,  $\Gamma_{\text{max}}$  is the maximum surface coverage, and  $K$  represents the equilibrium binding constant for the complexation (adsorption) of the guest ( $\text{Fc-COO}^-$ ) in an interfacial binding site ( $\beta$ -ACD). Plots of  $C\Gamma^{-1}$  vs  $C$  were linear (see Figure 6B, correlation coefficients in the range of 0.980–0.990), and least-squares analysis yielded the values for  $K$  and  $\Gamma_{\text{max}}$ , which are listed in Table 1.

As shown in Table 1, we prepared several types of Au/TOA/ $\beta$ -ACD-modified electrodes by changing the concentration of  $\beta$ -ACD in the "loading" solution and did, for each of these electrodes, the same set of experiments described above. As the concentration of  $\beta$ -ACD in the loading solution increases, a larger surface density of hosts is expected to be electrostatically held on the TOA monolayer surface. Our results show that as more  $\beta$ -ACD hosts are immobilized at the monolayer–solution interface, the measured value for the apparent binding constant with  $\text{Fc-COO}^-$  tends to increase. Although the magnitude of this trend is rather modest, its observation is in excellent agreement with the enhanced binding that is anticipated to result from the spatial accumulation of hosts.<sup>20</sup> All the  $K$  values measured were larger than the value obtained for this host–guest pair in homogeneous aqueous solution.<sup>17</sup> Our group has made similar observations in previous reports on interfacial binding.<sup>1a,b</sup>

Finally, the values for  $\Gamma_{\text{max}}$  given in Table 1 increase with the concentration of  $\beta$ -ACD utilized in the "loading" solution. All the values are clearly under the limiting value calculated for a compact monolayer of  $\beta$ -ACD hosts oriented with their main axes perpendicular to the interface ( $\sim 6 \times 10^{-11}$  mol/cm<sup>2</sup>). However, these  $\Gamma_{\text{max}}$  values reflect only indirectly the interfacial accumulation of  $\beta$ -ACD receptors, as they are just maximum surface excesses of  $\text{Fc-COO}^-$ . Nonetheless, our data suggest that the  $\beta$ -ACD hosts adsorbed on the carboxylate end of the self-assembled TOA molecules do not form a compact monolayer. Repulsive Coulombic interactions between neighboring  $\beta$ -ACD receptors may be responsible for their inability to self-assemble under these conditions into tightly packed monolayers. On the other hand, the loose assembly of the  $\beta$ -ACD hosts correlates well with the modest increase in the  $K$  values observed upon increasing surface density of the hosts. Since the degree of packing of the hosts is never too high, the observed binding enhancement due to the accumulation of binding sites is rather moderate throughout our experiments.

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- (18) To verify the adsorption of the positively charged  $\beta$ -ACD hosts on Au/TOA electrodes, we first recorded the cyclic voltammetric response of 1 mM  $\text{Ru}(\text{NH}_3)_6^{3+}$  in pH 8 boric acid buffer on these electrodes. The response was found to be essentially reversible ( $\Delta E_p = \sim 70$  mV), as anticipated, since the TOA monolayer does not act as an effective barrier to prevent the electron transfer to the cationic Ru(III) complex. Then, the electrode was immersed in 5 mM  $\beta$ -ACD (in pH 8 boric acid buffer) for 10 min, rinsed with pure water, and returned to the 1 mM  $\text{Ru}(\text{NH}_3)_6^{3+}$  solution, where the voltammetric response was monitored as a function of time. Immediately after transfer from the  $\beta$ -ACD solution, the measured potential difference between the cathodic and anodic peaks of the  $\text{Ru}(\text{NH}_3)_6^{3+}/2+$  couple was significantly larger ( $\Delta E_p = \sim 90$  mV) and was found to decrease slowly as time elapsed. After 10 min, the measured  $\Delta E_p$  value was the same as that recorded initially with the TOA-modified electrode, before immersion in the  $\beta$ -ACD solution. These data are consistent with the adsorption of  $\beta$ -ACD hosts on the Au/TOA electrode, as evidenced by the increased  $\Delta E_p$  value observed. However, the  $\beta$ -ACD hosts appear to undergo slow desorption, as suggested by the gradual return of the voltammetric response to that observed before the adsorption of the aminocyclodextrin receptors.
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