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Measurements of surface concentration and charge number per adsorbed molecule for a thiolipid monolayer tethered to the Au(111) surface by long a hydrophilic chain.

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Abstract:

The charge number per adsorbed molecule and surface concentration of 2,3-di-*O*-phytanyl-*sn*-glycero-1-octaethyleneglycol-D,L- α -lipoic acid ester (DPOL) at a Au(111) electrode surface was investigated by chronocoulometry using two different film deposition methods. First, a known amount of the thiolipid was transferred from the air-solution interface of a Langmuir trough onto the gold electrode surface via a Langmuir-Blodgett (LB) deposition. The charge density measurements for this monolayer system were used to determine the charge number per adsorbed DPOL molecule (electrosorption valency). The observed electrosorption valency values of the adsorbed DPOL film were much lower than the expected number of transferred electrons for a simple reductive desorption process. In the second deposition method, charge densities were measured for the electrode covered by a self-assembled DPOL monolayer. The electrosorption valency values determined from the LB DPOL film were used to calculate the packing density of the DPOL molecules within the self-assembled monolayer. The surface concentration of the molecules within the thiol monolayer with octaethylene glycol chains gave similar results to a related thiolipid with tetraethylene glycol chains (DPTL). This new finding

indicates that both of these molecules (DPTL and DPOL) assume a brush conformation in densely packed self-assembled monolayers.

Dedicated to the memory of Antonio Aldaz and in recognition of his contributions to electrochemistry

Introduction:

Knowledge regarding to the surface concentration of a chemisorbed self-assembled monolayer (SAM) is required for the development of new biosensors as well as for designing novel artificial biomembranes for biophysical and bioanalytical applications [1-10]. In the case of tethered biomembranes, it is important that the surface concentration of tethering molecules is known in order to understand the lateral packing density of the phospholipids in the artificial biomembrane. The extent of interaction between the tethered layer and electrode surface is also important when attempting to control the fluidity of a phospholipid bilayer. Alternatively, SAMs have also been implemented for modifying the hydrophilicity and reactivity of the electrode surface in order to promote the deposition of physisorbed phospholipid bilayers [10,11].

Historically, the so-called reductive desorption technique was introduced to measure the surface concentration of self-assembled thiols [12,13]. As the name implies, the reductive desorption method assumes that desorption of a thiol molecule from the surface occurs through a reduction reaction:

where $(RS - Au)_{ad}$ is the adsorbed thiol molecule, e^{-} is the electron, $(RS^{-})_{sol}$ is the thiolate ion desorbed into the solution phase and Au_{sur} is the adsorption site at the gold surface. The

reductive desorption technique relies on the measurements of charge, $\Delta\sigma$, flowing to the interface as a result of reaction 1. According to the Faraday Law, the charge is related to the surface concentration (Γ) of the thiol molecule by:

$$\Delta\sigma = nF\Gamma \quad [2]$$

where n is the number of electrons participating in the surface reduction reaction and F is the Faraday constant. However, Kunze *et al.*[14] and later Laredo *et al.*[15] demonstrated that the reductive desorption model which assumes a simple surface reduction reaction, is an oversimplification of the reaction at the metal-solution interface. This reductive desorption model overlooks the fact that the desorption process of the thiol involves a solvent substitution reaction described by:

and in addition, it consists of a change in the potential of zero total charge (E_{ptzc}). The solvent substitution constitutes an additional driving force for desorption of a thiol molecule as well as additional charge is flowing to the interface due to the change of the E_{ptzc} . To take these factors into account, the charge flowing to the interface during desorption of a thiol is given by $\sigma = -lF\Gamma$, where $l \neq n$ is referred to as the electrosorption valency or formal charge number per adsorbed molecule and can be defined as:

$$-l = \frac{1}{F} \left(\frac{\Delta\sigma}{\Delta\Gamma} \right) \quad [4]$$

Kunze *et al.*[14] derived a model that allows one to calculate the electrosorption valency and hence the surface concentration of adsorbed species within a SAM from experimental quantities with the help of the following equation:

$$l = \frac{(C_0 - C_1)}{F\Gamma} (E - E_{pzc}^0) - \frac{C_1 E_N}{F\Gamma} - n \quad [5]$$

where Γ is the surface concentration of the adsorbed thiol, C_0 and C_1 correspond to the capacitances of the film-free and film-covered gold (111) electrodes, respectively, E_{pzc}^0 is the potential of zero charge of the film-free electrode (since the measurements of the potential of zero charge of the film electrode are made in a nonadsorbing electrolyte, no distinction is made between potential of zero free and zero total charge, it is traditionally referred as pzc), E_N is the difference between the E_{pztc} of the covered and E_{pzc}^0 of the film-free electrode defined as $E_N = E_{pztc}^1 - E_{pzc}^0$. In a recent paper, Su *et al.*¹⁶ explained that σ is the total charge and showed the distinction between the meaning of the total and the free charge at the electrode surface covered by a monolayer of thiols.

Kunze *et al.*[14] investigated a SAM composed of a 2,3-di-*O*-phytanyl-*sn*-glycero-1-tetraethyleneglycol-D,L- α -lipoic acid ester (DPTL) thiolipid with a hydrophilic spacer, consisting of a tetraethylene glycol moiety, separating the hydrophobic phytanyl chains from the gold electrode surface. Schiller *et al* [5] designed this thiolipid to create a tethered bilayer on the metal surface where the first leaflet provided a water rich region between the metal and the hydrophobic membrane preventing denaturation of proteins reconstituted into the membrane. However, subsequent PM-IRRAS experiments by Leitch *et al* [13] and neutron reflectivity studies by Vockenroth *et al* [18] demonstrated that the tetraethylene glycol moiety of DPTL is coiled and the region between the phytanyl chains and the gold surface within the tethered bilayer contains only small amount of water. To solve this problem, Junghans and Koeper [19] investigated a series of thiolipids with variable length of the ethylene glycol (EG) spacer and

concluded that lipids with shorted chain EG form a better packed SAM but with a low content of water in the “hydrophilic” spacer. In contrast, lipids with longer chain EG form SAM with higher water content in the spacer region, however they display lower packing densities. Recently, Becucci et al [20] investigated 2,3-di-*O*-phytanyl-*sn*-glycero-1-octaethyleneglycol-D,L- α -lipoic acid ester (DPOL) as a tether lipid for supporting a tethered bilayer at a mercury electrode. This molecule is similar to DPTL, but has four additional ethylene glycol groups, which increase the length of the hydrophilic region compared to that of DPTL (Figure 1). Becucci et al. concluded that a bilayer assembled with DPOL has a larger water reservoir in the hydrophilic spacer region than that made of DPTL, but appeared to be somewhat leakier. The results of their study demonstrated that tethered DPOL bilayers may act as a good system for further biomimetic research.

The goal of this paper is to apply the methodology developed by Kunze et al [14] to determine the surface concentration of DPOL molecules self-assembled at a gold electrode surface. This study will compare the properties of DPOL and DPTL SAMs in order to assess the influence of the hydrophilic spacer length on the packing density of the molecules in these monolayers. This study was made in preparation of future IRRAS investigations that are focused on gaining a better understanding about the effect of the ethylene glycol spacer length on the conformation and hydration of the SAM of DPOL.

Figure 1: The molecular structure of DPTL ($n = 4$) and DPOL ($n = 8$).

Experimental:

The 2,3-di-*O*-phytanyl-*sn*-glycero-1-tetraethyleneglycol-D,L- α -lipoic acid ester (DPTL) and 2,3-di-*O*-phytanyl-*sn*-glycero-1-octaethyleneglycol-D,L- α -lipoic acid ester (DPOL) were synthesized and characterized in Schwan laboratory at the University of Guelph [21]. The Au(111) electrode, with a surface area of 0.172 cm², was fabricated in-house as described in [22]. A standard three-electrode configuration was implemented with a gold (111) working electrode, a coiled gold wire counter electrode and a saturated calomel reference electrode (SCE) in all electrochemical experiments. The electrochemical instrumentation included a HEKA PG590 Potentiostat/ Galvanostat (HEKA Electronics, Chester, NS, CA), 7265 DSP Lock-In Amplifier (EG&G Instruments, Princeton, NJ, USA) and a NI-DAQ 6052E data acquisition interface (National Instruments, Vaudreuil-Dorion, QC, CA) was used to digitize the analog signals from the working electrode for computer compatibility and visual display. All potentials of the collected electrochemical data reported in this work have been converted to the Ag/AgCl in saturated KCl reference electrode scale (+45 mV vs SCE for comparative purposes).

All glassware, including the electrochemical cell, was cleaned in a hot mixture of concentrated H₂SO₄: HNO₃ = 3: 1 v/v for 1.5 hours. The glass components were then rinsed with copious amounts of ultrapure, 18.2 M Ω cm, Milli-Q water (EMD Millipore Canada, Etobicoke, ON, CA). The glassware was then soaked in the ultrapure water for at least 12 hr and rinsed again with Mill-Q water prior to use. Sodium fluoride (>99%) was purchased from Sigma-Aldrich (Oakville, ON, CA). Appropriate amounts of the solid NaF were weighed and cleaned for 20 min in a UVO cleaner (Jelight, Irvine, CA, USA) prior to electrolyte preparation. The

cleaned NaF was then dissolved in ultrapure water to obtain a final solution concentration of 0.1M.

The stock SAM solutions were prepared by dissolving either DPTL or DPOL in a known volume of chloroform to give a final concentration of 1 mg mL⁻¹. The single crystal facet of the Au(111) electrode was freshly flame-annealed and cooled before incubating for approximately 12 hours in the appropriate self-assembly solution to ensure completion of a densely packed monolayer. The SAM-modified Au(111) electrode surface was thoroughly rinsed with HPLC grade methanol to remove any non-chemisorbed molecules from the surface. Copious amounts of ultrapure Milli-Q water was then used to rinse any residual methanol from the modified electrode surface. The electrode was dried under a stream of Ar_(g) and placed in the electrochemical cell containing 0.1 M NaF_(aq) for electrochemical measurements.

The second method of electrode modification was performed using the Langmuir–Blodgett deposition. First, the gold electrode was submerged into the water sub-phase prior to deposition of the Langmuir film. Secondly, approximately 40 µL of 1 mg mL⁻¹ DPOL stock solution was dispersed onto the surface of the Milli-Q water subphase in a dropwise manner. The chloroform was allowed to evaporate from the water surface for 15 min after which the barriers were closed to obtain a constant surface pressure of 35 mN m⁻¹. Finally, the electrode was then withdrawn vertically through the monolayer film while maintaining a constant surface pressure placing a monolayer of known concentration onto the electrode surface. The Langmuir-Blodgett (LB) monolayer of DPOL was deposited onto the Au(111) surface using freshly prepared stock solution to ensure that an accurate surface concentration was obtained for each series of depositions. A transfer ratio of 1.0 ± 0.1 was accepted as a successful transfer of the DPOL from

the air/ water interface to the electrode surface. The electrode was then allowed to air dry for approximately 2 hours before being placed into the electrochemical cell containing 0.1 M NaF.

After the modified electrode was placed in the electrochemical cell, the electrolyte solution and atmosphere above the solution were deaerated with $\text{Ar}_{(\text{g})}$. The electrochemical circuit was then closed by forming a hanging meniscus contact between the electrode surface and electrolyte solution. Differential capacitance measurements were performed in a potential range from $(-1.2 \leq E \leq 0.45) \text{ V}_{\text{Ag}/\text{AgCl}}$. The surface charge densities at the Au electrode were measured using the procedure described in details in [14, 15]. The measurements were divided into two potentials regions. The first was from: $(-1.15 \leq E \leq -0.65) \text{ V}_{\text{Ag}/\text{AgCl}}$ in 0.1M NaF solution. By stepping the potential from a the value E to -1.15V we were able to determine the charge density difference for either the full monolayer covered electrode at -0.65V or partially covered electrode for $E < -0.65\text{V}$ and $E = -1.15\text{V}$. The monolayer is totally desorbed at $E = -1.15 \text{ V}$. The value of the charge density for the electrode in the supporting electrolyte without the film was used to convert the charge density difference into the absolute charge densities. The electrodes were flame annealed and fresh monolayers were deposited onto the Au(111) surface for each charge density measurement.[14] The second region $(-0.65 \leq E \leq +0.45) \text{ V}_{\text{Ag}/\text{AgCl}}$ corresponded to potentials where the monolayer was stable at the electrode surface. These measurements were performed in 0.1M NaF acidified with an addition of HF to pH=5.5 to suppress the onset of OH adsorption. The potential was stepped from the value E to $E = -0.65 \text{ V}$ and the charge density was determined. The absolute value of the charge density at $E = -0.65 \text{ V}$ determined in the first region was used to convert the charge density difference into the absolute value of the charge density. The charge densities were measured at 0.050 V increments within the potential intervals listed above. The charge densities for the supported electrolyte were measured by stepping the potential from a

value E to the final potential $E=-1.15$ V. The potential of zero charge $E=0.300$ V determined independently from a minimum on the differential capacity curve in a diluted electrolyte was used to convert the measured charge density difference into the absolute charge density for the supporting electrolyte.

The immersion method described by Ramirez *et al.* [23] and Su *et al.*[16] was implemented in the measurement of the potential of zero free charge (E_{pzfc}). A flame-annealed Au(111) electrode or a freshly prepared thiol SAM-modified Au(111) electrode was placed in the electrochemical cell above the electrolyte. The electrode was connected to the potentiostat and polarized to a series of potentials at which the adsorbed thiol was stable on the Au(111) surface. The electrode was slowly lowered toward the electrolyte surface and the immersion current, flowing to the electrode when it was brought into contact with the electrolyte, was recorded. The charge for building up the electrochemical double layer was then calculated by integrating the immersion current transients. Each data point was collected in triplicate, averaged and plotted against the potential at which it was measured. A linear regression of the data was then used in order to interpolate the value of the potential of zero free charge (E_{pzfc}).

Results and Discussion:

Charge Density Studies of DPOL Monolayers:

Two methods were used to form DPOL monolayers at the gold electrode surface. The first employed Langmuir-Blodgett transfer of a monolayer spread at the air-solution interface of a Langmuir trough. The compression isotherm of DPOL is shown in Figure 2. For comparison, the compression isotherm for DPTL is also plotted in this figure. The shape of the isotherms indicates that both monolayers are in the liquid expanded state. For DPOL, compression of the

monolayer begins at $\sim 350 \text{ \AA}^2$ while for DPTL, the compression starts much later at a value of $\sim 225 \text{ \AA}^2$. The DPTL monolayer starts to collapse at about 35 mN m^{-1} while the DPOL monolayer starts to collapse at higher surface pressure $\sim 41 \text{ mN m}^{-1}$ suggesting that in the compressed state, DPOL monolayers are somewhat more stable. During the compression of these monolayers the molecules undergo a progressive transition from the “pancake” to “mushroom” and finally to “brush” conformations. The longer polyethylene glycol chain allows the DPOL molecule to have much higher footprint in the “pancake” and “mushroom” states. However, at higher surface pressures, the footprints of DPTL and DPOL molecules are approximately the same in the “brush” state. At a pressure of approximately 35 mN m^{-1} , a tightly packed monolayer of DPOL was formed at the air-water interface with a mean molecular

Figure 2: Compression isotherms of DPOL and DPTL at the air/ water interface. A constant pressure of 35 mN m^{-1} was selected as the transfer pressure to ensure a tightly packed DPOL monolayer was deposited onto the Au(111) electrode surface.

area of approximately $110 \text{ \AA}^2$, corresponding to a surface concentration of $1.5 \times 10^{-10} \text{ mol cm}^{-2}$. This pressure was selected as the LB transfer pressure for depositing a well packed monolayer on to the Au(111) electrode surface. For comparison in ref 14 the monolayer of DPTL was transferred at the film pressure of 30 mNm^{-1} and the mean molecular area $112 \text{ \AA}^2$. The average transfer ratio of the LB film of (DPOL) deposited on the gold electrode was 0.95 ± 0.07 . As a result, the average actual surface concentration on the electrode was $1.4 \pm 0.15 \times 10^{-10} \text{ mol cm}^{-2}$. This value will be used to calculate the electrosorption valency of DPOL molecules in the film at the Au surface.

Figure 3: A) Charge densities and B) charge density differences between the modified and unmodified gold electrode for LB deposited (○) and self-assembled (●) DPOL monolayer films, measured in a 0.1 M $\text{NaF}_{(\text{aq})}$ electrolyte

The open circles in Figure 3a, plot the charge density curve for the Au(111) electrode covered by a DPOL monolayer that was deposited using the LB method. The black squares in that panel represent the charge density curve of the Au(111) electrode measured in a 0.1 M NaF

electrolyte in the absence of a monolayer film. These two charge density curves were used to calculate the charge density difference between the DPOL monolayer-covered and the monolayer-free gold electrode surfaces denoted by open circles in Figure 3b. The monolayer of DPOL was also formed at the gold electrode by self-assembly from the 1 mg/mL solution of DPOL in chloroform. The charge density curve for the electrode covered by this SAM and the difference between charge densities for the DPOL covered and the film-free electrodes are plotted as closed circles in Figure 3a and 3b, respectively. The charge densities for the electrode covered by SAM and the corresponding $\Delta\sigma$ are larger for the electrode covered by the SAM compared to the monolayer transferred using the LB method. These differences indicate that the monolayer formed by self-assembly has a higher packing density.

The immersion method [16,23] was employed to measure the potential of zero free charge of the gold electrode covered by the SAM. Figure 4 plots the charge densities determined by integration of the current measured when the SAM covered electrode was brought in contact with the electrolyte at a controlled potential indicated in this figure. The linear regression of this data gives the value of the potential of zero free charge $E_{pzfc} = 0.19 \pm 0.02 \text{ V}_{\text{Ag}/\text{AgCl}}$. This is the potential at which the electrostatic charge at the SAM covered electrode is equal to zero. It is important to note, the E_{pzfc} is different than the potential of zero total charge, E_{pztc} , which as Figure 3a shows is equal approximately to $-0.638 \text{ V}_{\text{Ag}/\text{AgCl}}$. The E_{pztc} corresponds to potential where the free energy of the SAM-covered Au(111) electrode has a maximum. For comparison, Figure 4 plot also the absolute charge density data determined earlier from chronocoulometric experiment and shown in Figure 3A. The two sets of data have similar slope of $\sim 3 \pm 0.3 \mu\text{F cm}^{-2}$.

We emphasize that DPOL is an electrostatically neutral dithiol which structure is shown in Figure 1. Its adsorption involves a breaking of the S-S bond and formation of Au-S, Au-S

bonds. No oxidation reaction takes place during DPOL adsorption in contrast to the oxidative adsorption of monothiolates. In fact the dithiol is an oxidized form of the dithiolate formed during desorption at negative potential. No charging of the interface takes place during the SAM formation. Consequently, the electrode is not charged before contacting the solution in the immersion method to measure the free charge for the SAM covered electrode.

Figure 4. Left axis and red points charge densities determined by integration of the current flowing to the electrode when the DPOL SAM covered Au(111) electrode was brought in contact with the 0.1M NaF acidified with HF to pH=5.5 solution at the plotted applied electrode potentials. Right axis and black points, absolute charge densities determined from the chronocoulometric experiment and plotted earlier in Figure 3A.

Following the procedure described in [14,15], equation 4 can be used to calculate the electrosorption valency, Γ , for DPOL molecules adsorbed at the electrode surface, using the $\Delta\sigma$ for the electrode with the monolayer transferred by the Langmuir-Blodgett method and the surface concentration of DPOL transferred onto the electrode surface ($1.4 \pm 0.2 \times 10^{-10}$ mol

cm^{-2}). The calculated values of l are plotted as a function of the electrode potential in Figure 5. It should be noted that the values of l deviate significantly from $n = 2$, which is expected for a

Figure 5. Open circles plot the electroadsorption valency for the LB deposited DPOL adsorbed monolayer at the electrode surface calculated using the Equation 4 and the charge density difference in Figure 3b. Closed circles display the calculated l values for the SAM covered electrode using Equation 5. Ten percent error applies to the values plotted in this diagram. The error bars are not shown for the sake of the clarity of presentation.

dithiol. The closed circles in Figure 5 display electroadsorption valencies calculated from Equation 5 for the water substitution model using the experimental charge data for the DPOL SAM at the gold surface. The capacitances, C_0 and C_1 , were determined from the slopes of the charge density plots in Figure 3a; $C_1 E_N = -\Delta\sigma(E_{pzc}^0)$ and the surface concentration (Γ) for the SAM, which is equal to $2.3 \pm 0.2 \times 10^{-10} \text{ mol cm}^{-2}$ as shown in Figure 6. We note that an extrapolation of the linear fragment of the charge density curve for the SAM covered surface

from the value of the charge at the pzc of the film free electrode to zero charge density gives:

$E_N = E_{pzc}^1 - E_{pzc}^0$. The SAM is desorbed before E_{pzc}^1 is reached. However, one does not need to determine E_{pzc}^1 . A simple geometric consideration shows that the negative of the charge density for the SAM covered electrode at E_{pzc}^0 is equal to the product of E_N and C^l (the slope of the charge density plot). Consequently, the $C_1 E_N = -\Delta\sigma(E_{pzc}^0)$ and this term can be read directly from the experimental charge density plot.

The excellent agreement between the electrosorption valencies determined from the data of the monolayer transferred by the LB method and that calculated for the SAM using the equation derived from the water substitution model indicates that the model can accurately describe the potential dependence of the electrosorption valency of these thiol molecules. It should be clarified that the surface coverage Γ determined with the help of electrosorption valency obtained using the LB film is used in equation 5. However, this is a constant number. It does not contribute to the potential dependence of the calculated value. The agreement between the electrosorption valency obtained for the LB film and the electrosorption valency calculated for SAM with the help of equation 5 explains that the potential dependence of l is due to the solvent displacement and the shift of the potential of zero charge. Its significance is that it shows why the electrosorption valency is a potential dependent value, rather than a constant number assumed in the reductive desorption method [12,13]

Next, equation 4 was employed to calculate the surface concentration of DPOL in the self-assembled monolayer using the values of l determined for the monolayer transferred by the LB method and $\Delta\sigma$ determined for the SAM. The calculated values are plotted as a function of potential in Figure 6. Their changes with potential are within the experimental uncertainties and

the average value is $2.3 \pm 0.2 \times 10^{-10} \text{ mol cm}^{-2}$ (open squares) or the area per molecule $72 \pm 8 \text{ \AA}^2$ (solid squares). Within experimental error, these numbers agree with the surface

Figure 6. Surface concentration of DPOL in self assembled monolayer determined using $\Delta\sigma$ for the SAM and l for the monolayer transferred using the LB method.

concentration of the SAM of DPTL, which had an average molecular area of $81 \pm 9 \text{ \AA}^2$ for potentials ranging from $-0.7 < E < 0.25 \text{ V}_{\text{Ag/AgCl}}$ [14]. The structural differences between DPOL and DPTL consist of a change in the length of the tether which contains eight ethylene glycol units in DPOL and four in DPTL. The fact that the two molecules have similar footprint in the SAMs indicates that at gold surface the tethers assume brush conformation. This result is consistent with compression isotherms in Figure 2, showing that similar areas per molecule are also observed at higher surface pressures, when the molecules are in the “brush” state at the air/water interface.

Figure 7 compares differential capacitance curves measured for the Au(111) surface covered by SAMs of DPOL and DPTL. The capacitance of the electrode covered by the SAM of DPOL is much higher than the electrode with the SAM of DPTL. This behavior indicates that although the packing densities for the two thiols are similar the SAM of DPOL contains more defects. A minimum capacitance of $2.5 \mu\text{F cm}^{-2}$ for the DPOL-covered electrode is observed at the most positive potentials and will be referred to as state I of the film. This value is in a reasonable agreement with the slope of the data in Fig 4 corresponding to $\sim 3 \pm 0.3 \mu\text{F cm}^{-2}$. The direct measurement of the differential capacitance gives a more precise value than the slope of the charge density data. The capacitance increases to a value of $\sim 6.5 \mu\text{F cm}^{-2}$ at potentials more negative than $-0.2 \text{ V vs Ag/AgCl}$. This corresponds to

Figure 7: Differential capacitance curves measured when potential is scanned in negative direction at sweep rate of 5 mV s^{-1} for the film free Au(111) (dotted-line), and covered by the SAM of DPTL (blue line) and DPOL SAM (red line) electrode in contact with $0.1 \text{ M NaF}_{(\text{aq})}$ electrolyte.

state II of the film. In Figure 7, the top horizontal axis plots the rational potential ($E - E_{\text{pzfc}}$), which is a measure of the electrostatic potential acting on the monolayer. It shows that state I of the

film is observed at positive electrostatic potentials and state II at negative potentials. The transition from state I to state II takes place in the vicinity of the E_{pzc} . The capacitance for DPTL coated electrode is equal to $0.8 \mu\text{F cm}^{-2}$ at positive end of potentials. However, it increases slightly when potential decreases from +0.4 to -0.6V vs Ag/AgCl [14,24,25]. The PM IRRAS experiments by Leitch et al [17] demonstrated that the increase in capacitance correlates with the increase of the water content in the tetraethylene glycol fragment of the monolayer. We are performing similar PM-IRRAS experiments to provide molecular level information about the difference in the structure of the DPOL monolayer in the two states. They will be described in the next publication.

Summary and Conclusions:

Chronocoulometric experiments were performed on monolayers of DPOL (a thiolipid with octaethylene glycol spacer) deposited at the Au(111) surface using Langmuir-Blodgett and self-assembly techniques. The LB method allowed us to transfer a pre-determined amount of DPOL molecules onto the gold surface. The chronocoulometric measurements provided the difference in charge density between electrodes covered and free of this monolayer, allowing us to determine the electrosorption valency (charge number per adsorbed molecule). The values of the electrosorption valency deviated significantly from the number expected for a simple reductive desorption of this dithiol. Independent chronocoulometric measurements were performed on the electrode coated by a monolayer of DPOL self-assembled at gold electrode surface. The difference in charge densities and electrosorption valencies determined from the first experiment were then used to calculate the surface concentration of DPOL in the self-assembled monolayer. The surface concentration of the DPOL SAM was similar to the surface concentration in a SAM of DPTL (a thiolipid with tetraethyleneglycol spacer) determined earlier

[14]. The results indicate that the two molecules assume a brush conformation in the self-assembled monolayers implying that the length of the polyethylene glycol spacer has little effect on the overall packing density of the thiolipid. The model based on the competitive adsorption of the thiolipid and water was used to predict changes of the electrosorption valency with electrode potential. The values calculated from this model were in excellent agreement with electrosorption valencies determined for the monolayer deposited by the LB method. The results of this paper are significant for the development of biomimetic membranes. They also illustrate the success of the chronocoulometric method that is first applied to the LB monolayer to determine the electrosorption valencies and then is using these values to calculate packing density in a monolayer formed by self-assembly.

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