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QUANTITATIVE SUBTRACTIVELY NORMALIZED INTERFACIAL FOURIER TRANSFORM INFRARED REFLECTION SPECTROSCOPY STUDY OF THE ADSORPTION OF ADENINE ON AU (111) ELECTRODES

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ABSTRACT

Quantitative subtractively normalized interfacial Fourier transform infrared reflection spectroscopy (SNIFTIRS) was used to determine the molecular orientation and identify the metal-molecular interactions responsible for the adsorption of adenine from the bulk electrolyte solution onto the surface of the Au (111) electrode. The recorded p-polarized IR spectra of the adsorbed species were subtracted from the collected s-polarized IR spectra to remove the IR contributions of the vibrational bands of the desorbed molecules that are located within the thin layer cavity of the spectroelectrochemical cell. The intense IR band around 1640 cm^{-1} , which is assigned to the pyrimidine ring stretching vibrations of the C5-C6 and C6-N10 bonds, and the IR band at 1380 cm^{-1} , which results from a combination of the ring stretching vibration of

the C5-C7 bond and the in-plane CH bending vibration, were selected for the quantitative analysis measurements. The transition dipoles of these bands were evaluated by DFT calculations. Their orientations differed by $85 \pm 5^\circ$

The tilt angles of adsorbed adenine molecules were calculated from the intensity of these two vibrations at different potentials. The results indicate that the molecular plane is tilted at an angle of 40° with respect to the surface normal of the electrode and rotates by 16° around its normal axis with increasing electrode potential. This orientation results from the chemical interaction between the N10 and gold atoms coupled with the π - π parallel stacking interactions between the adjacent adsorbed molecules. Furthermore, the changes in the molecular plane rotation with the electric field suggest that the N1 atom of adenine must also participate in the interaction between the molecule and metal.

INTRODUCTION

The adsorption of DNA bases at solid electrodes is an interesting area of research because the electrode/electrolyte interface can be used to model the electrostatic properties of wide variety of biological interfaces. These studies offer a unique opportunity to investigate the effect of the electric field on the organization of DNA bases and the interactions between their complementary DNA bases. Moreover, these systems have technological interest in the development of new biocompatible materials, biosensors and molecular electronic devices, in addition to gaining a better understanding of the fundamental principles of molecular recognition.^{1, 2, 3, 4, 5, 6}

The adsorption of adenine on gold single crystal electrodes of low Miller index planes (Scheme 1) was previously characterized by cyclic voltammetry and capacitance measurements,⁷ which demonstrated that the adenine molecules are weakly chemisorbed to the surface gold atoms and that the adsorption process is strongly affected by the pH of the bulk and the crystallographic orientation of the metal surface.⁸ In addition, there is also a close relationship between the chemisorption of adenine and the lifting of the reconstruction of the electrode surface. The thermodynamic study of the adenine adsorption on the Au(111) surface from neutral solutions was performed using charge density data, which was obtained by integrating of the chronoamperometric curves.⁷ The current transients were measured by employing a specially designed potential-step program to avoid the influence of the surface reconstruction processes on the total charge.^{9, 10} The value for the free Gibbs energy of adsorption (-35 kJ mol^{-1}), obtained via thermodynamic analysis of the charge density data, further supported a weak chemisorption process. The maximum surface excess ($3.5 \times 10^{-10} \text{ mol cm}^{-2}$) suggested that the molecular plane of adenine adopts a tilted orientation with respect to the surface.⁷

Potential controlled Infrared Reflection Absorption Spectroscopy (IRRAS)^{8, 11, 12} and scanning tunneling microscopy (STM),¹³ were performed in order to obtain microscopic information about the adsorption of adenine on the surface of gold electrodes, such as the nature of intermolecular interactions, orientation of the adenine molecules, in addition to gaining a better understanding about how the molecule is coordinated to the gold atoms on the surface. The electrochemical STM measurements confirmed that adenine induces the lifting of the surface

reconstruction. The images show that the gold atoms released by lifting the reconstruction of the Au(111) surface form islands of a monoatomic height that remain “frozen” along the characteristic Au(111) reconstructed solitons (reconstruction lines). The images also showed that the adenine ad-layer is organized into twisted rows, which are aligned along the three main directions of the Au(111) surface, $[\bar{1}, 1, 0]$, $[1, 0, \bar{1}]$ and $[0, \bar{1}, 1]$. Each row is formed by molecules stacked every 3-4 angstroms, which is compatible with π -electronic intermolecular interactions.

The IRRAS studies of the electrosorption of adenine have provided information about the chemical identity of the adsorbed species and nature of the adenine coordination to the metal surface.^{8, 11, 12} The IR absorption spectra of adsorbed adenine on gold electrodes display bands that correspond to the in-plane vibrations of the pyrimidine ring. The appearance of this band suggests that adenine adopts a normal or tilted orientation of the molecular plane relative to the electrode surface according to the surface selection rules. The assignments of the observed surface active IR bands in comparison with the spectra published for adenine-metal complexes in the literature, suggests that adenine interacts with the gold electrode via the amine nitrogen atom (N10), in combination with either the pyrimidinic nitrogen (N1) or the imidazolic nitrogen (N7) as presented in Scheme 1. Moreover, the analysis of the spectroelectrochemical data as a function of pH,^{8, 11} lead to the conclusion that only the neutral adenine molecule and the N9-deprotonated anionic adenine form can chemically interact with the metal, even at very low pH where adenine is in its cationic (i.e. protonated at the N1 atom) form. These results imply that the chemical

adsorption of adenine induces the deprotonation of the N1 atom, even at pH values that are significantly lower than the corresponding pKa value of 4.8.^{14, 15}

Unfortunately, the analysis of the IRRAS data was qualitative in nature; therefore, the conclusions were merely speculative. Consequently, there are many questions that remain unclear about the structure of the adsorbed adenine layer on the surface of the gold electrodes, which include: How do the N1 or N7 atoms of adenine participate in the interaction with the electrode? What is the actual tilt angle of the molecular plane relative to the electrode surface? How does the tilt angle change with the electrode potential? What is the driving force for this change? Moreover, in the presence of thymine (the complementary base of adenine),^{16, 17} the infrared absorption bands suggest that there is a change in the overall orientation of the adsorbed adenine, which may result in a variation in the coordination between adenine and the gold atoms on the electrode surface. In order to determine changes in the molecular orientation, a quantitative evaluation of the tilt angles of the adsorbed adenine is required. In the last decade, several studies have applied SNIFTIRS to gain quantitative information about electrochemically adsorbed layers on single crystal electrodes, which include pyridine on Au(110),¹⁸ citrate on Au(111),¹⁹ 2-mercaptobenzimidazole on Au(111),^{20, 21} or sulfate on Pt(111).²² All these studies are based on the comparison between the experimental SNIFTIR spectrum of the adsorbed molecular film and a theoretically simulated spectrum of randomly oriented molecules with the same surface concentration.²³ The integrated intensity of an IR absorbance band of the adsorbed molecular film obtained with p-polarized light, $A_{p(surface)}$, where the electric field of the photon is parallel to the plane of incidence, is proportional to

the scalar product of the transition dipole vector and the electric field of the photon according to the following expression:^{24, 25}

$$\int A_{p(\text{surface})} d\bar{\nu} \propto \Gamma \left| \vec{\mu} \cdot \vec{E}_p \right|^2 = \Gamma |\mu|^2 \langle E_p^2 \rangle \cos^2 \theta \quad (1)$$

where Γ is the surface concentration of the IR absorbing species, θ is the angle between the transition dipole moment and the electric field vector of photon, $|\mu|$ is the transition dipole modulus and $\langle E_p^2 \rangle$ is the mean square electric field strength of the p-polarized radiation at the reflection surface. Equation 1 can also be used to calculate the orientation of an adsorbed film of the same surface concentration with randomly oriented molecules, however, the angle of the transition dipole moment in this case would be equal to the magic angle ($\theta = 55^\circ$). From the ratio of the integrated intensities of the experimental and calculated spectra for randomly adsorbed molecules, it is possible to determine the angle of the transition dipole moment, θ , for the adsorbed adenine on the gold surface.

$$\frac{\int A_{\text{exp}} d\bar{\nu}}{\int A_{\text{calc}} d\bar{\nu}} = \frac{\cos^2 \theta}{\cos^2 55^\circ} \quad (2)$$

Knowing the direction of the transition dipole with respect to the molecular coordinates, it is then possible to attain the average orientation of adsorbed molecules on the metal surface.^{24, 25, 26}

The aim of this work is to perform the quantitative SNIFTIRS study of adenine adsorption on the Au(111) electrode from a neutral solution to determine the orientation of adsorbed adenine molecules on the electrode surface. The molecular level information will be used to determine which fragments of the adenine molecule

(NH₂ group and/or either N1 or N7 heteroatom) are coordinated to the electrode surface and the average tilt angle of the adenine molecular plane with respect to the surface normal. Furthermore, we will show how the electrode charge influences the orientation of molecules at the electrode surface. This work demonstrates the power of quantitative SNIFTIRS studies of organic films at an electrode surface by illustrating that the spectroscopic data not only provides unique information regarding structure of the film, but also gives insight into how the molecules are coordinated to the metal surface.

EXPERIMENTAL

Reagents and electrodes.

The sodium fluoride (ACS certified, Fisher Scientific) was used as the supporting electrolyte. It was cleaned for 30 minutes in an ozone chamber (UVO cleaner, Jelight, Irvine, CA) prior to use. The adenine (Sigma-Aldrich) was used without further purification. The solutions were prepared from deuterium oxide, D₂O (99.9%, Cambridge Isotope Laboratories, Cambridge, MA). The working electrode was a gold Au(111) oriented single crystal, prepared following the Clavilier method²⁷. It was flame annealed before its insertion into the spectroelectrochemical cell. A platinum foil was used as counter electrode and a silver/silver chloride reference electrode. A salt bridge, containing the electrolyte solution, was used to avoid interference from chloride ions present in the reference electrode. The analyte solution in the infrared reflection absorption measurements consisted of 2 mM adenine in 0.5 M NaF/D₂O. In the transmission measurements, an adenine concentration of 10 mM was dissolved in

the 0.5 M NaF/D₂O electrolyte. Prior to all experiments, the IR cell was cleaned in an acid bath (1:3 mixture of HNO₃ and H₂SO₄) and thoroughly rinsed with Milli-Q UV plus (Millipore, Bedford, MA) water ($\geq 18.2 \text{ M}\Omega \text{ cm}$). The IR cell was soaked in Milli-Q water for several hours, rinsed again and then dried over night. A HEKA potentiostat (Lambrecht/Pfalz, Germany) was used to apply the desired potential to the cell. The spectra were recorded with a resolution of 4 cm^{-1} (i.e. spectral data points are computed every 2 cm^{-1}). The bands are plotted by interpolation between these points; therefore, the band center position can be determined with uncertainty 0.5 cm^{-1} .²⁸

FT-IR measurements.

All of the FT-IR spectra have been obtained with a Nicolet 8700 spectrometer equipped with a liquid N₂ cooled MCT-A detector. The instrument and tabletop optics module (TOM) were purged with dry CO₂-free air from a Puregas heatless dryer (Whatman, Piscataway, NJ). Infrared transmission spectra of the aqueous (H₂O and D₂O) adenine solutions (10 mM) in 0.5 M NaF were measured with a transmission cell that consisted of two plane calcium fluoride windows spaced by a c.a. 50- μm PTFE gasket. The exact thickness of the transmission cell was determined from the separation between constructive interference fringes of the transmission spectrum registered in air.²⁹

For the infrared reflection absorption experiments, the IR beam was polarized in either the plane of incidence (p-radiation) or the direction perpendicular to the plane of incidence (s-radiation). According to the surface selection rules for metals,³⁰ the intensity of the s-polarized radiation is cancelled at the reflection plane, which

means that only species that are desorbed in the bulk solution of the thin layer cavity between the metal and IR prism are capable of absorbing the infrared radiation. In contrast, the IR absorption of the p-polarized radiation occurs at both the metal electrode surface and in the bulk solution. Furthermore, the electric field of the p-polarized radiation is enhanced upon reflection at the metal surface, which means that p-polarized radiation is more sensitive to surface species than those desorbed in the bulk of solution.

The design of the spectroelectrochemical IR cell has been previously described in the work by Li et al.¹⁸ The optical set up, according to the design of Faguy,^{31, 32, 33, 34} allows for adjustments of the incidence angle and focal point of the IR beam. The IR transparent window was a ZnSe hemispherical prism with a diameter of 25 mm. The Au(111) surface was pressed against the IR window to create a thin layer of electrolyte with a thickness of a few micrometers. In order to get the maximum surface enhancement of the p-polarized radiation at the reflection point, the incidence angle of the IR beam has been set just below the critical angle with a focal distance between 9 and 10 mm from the base of the ZnSe hemisphere prism. The incidence angle of the IR beam and thickness of the electrolyte layer between the optical window and the metal surface have been precisely determined with the Hansen model³⁵ where the experimental reflectivity spectrum is compared with a theoretical spectrum that is calculated for three parallel, homogeneous phases (ZnSe | electrolyte | Au) according to the method developed by Li et al.¹⁸

The SNIFTIR spectra were obtained by collecting the sample reflectivity spectrum (R_{ads}) with adsorbed adenine by registering 50 interferograms at a sample

potential (E_{ads}) at which adenine molecules are adsorbed to the gold surface, and the reference reflectivity spectrum (R_{des}) by registering the same number of interferograms at a low potential (E_{des}) where adenine is fully desorbed from the electrode surface. This collection cycle has been repeated 80 times and the results were averaged in order to increase the signal to noise ratio. The corresponding IR reflection absorption spectrum at a single sample potential is obtained representing $\Delta R/R_{des}$ vs. wavenumber:

$$\left(\frac{\Delta R}{R_{des}}\right)_{SNI} = \frac{R_{des} - R_{ads}}{R_{des}} \approx 2.3\Gamma[\varepsilon(E_{des}) - \varepsilon(E_{ads})] = 2.3\Delta A \quad (3)$$

Where Γ is the surface concentration of the adsorbed species, $\varepsilon(E_{des})$ and $\varepsilon(E_{ads})$ are the molar absorptivity coefficients of adenine in the completely desorbed and adsorbed states, respectively. As a result, the negative bands in the SNIFTIR spectrum correspond to the IR absorption by molecules that are adsorbed at the electrode surface, while positive bands account for an increase in IR absorption of the molecules located within solution. This increase in IR absorbance is caused by a decrease in the bulk solution concentration due to an adsorption process. The loss in IR absorbance occurs if there is a shift in the frequency of the IR band or if the adsorbed molecule adopts an orientation where the direction of the transition dipole moment for the given vibration is parallel to the metal surface.

The sample potential, E_{ads} , was changed within the limits of adsorption of adenine. The SNIFTIRS measurements, including the potential steps and collection of the interferograms, were driven by the macro utility program within the OMNIC™ Spectral Software package (Thermo Scientific, Waltham, MA, USA) on a computer

equipped with a digital to analog converter (PIO DA4 board, ICP DAS CO,Hsinchu, Taiwan).

DFT calculations of the vibrational transitions.

The directions of the transition dipole of each vibration have been obtained by DFT geometry optimization using a Unix version of Gaussian 09 on a Fujitsu Primergy cluster. The vibrational calculations were performed with the B3LYP/ 6-31++G(d,p) functional and basis set as previously described by Giese and McNaughton in their study of adenine adsorption on silver surfaces³⁶.

RESULTS AND DISCUSSION

FT-IR spectra of adenine in solutions of neutral pH.

Figure 1 displays the IR transmission spectra of the 1800 – 1300 cm⁻¹ region for 10 mM adenine/0.5 M NaF electrolyte solutions prepared in D₂O (Figure 1a) and H₂O (Figure 1b). All of the spectral bands within this frequency range correspond to the *in-plane* vibrations, which are associated with the NH₂ scissoring and skeletal stretching vibrations.³⁶

- Figure 1 -

The hydrogen atoms that are associated with the nitrogen groups of adenine readily exchange with deuterium from the 0.5 M NaF/D₂O electrolyte. The IR absorbance for the deuterated adenine shows the appearance of an intense band around 1630 cm⁻¹, which is associated with the in-plane pyrimidine ring stretching

vibrations of the C5-C6 and C6-N10 bonds. In the H₂O electrolyte, the pyrimidinic ring stretching band (1639 cm⁻¹) overlaps with the scissoring vibrational band (1652 cm⁻¹) of the amine group. In deuterium oxide, the bending vibrational mode of the amine group is shifted to lower frequency (~1200 cm⁻¹), while the pyrimidinic ring stretching vibration remains within the 1600 cm⁻¹ region, which completely resolves these two independent vibration bands. Additionally, the strong OH bending mode of water is also active in the 1600 cm⁻¹ region whereas, the OD bending mode is shifted to 1200 cm⁻¹. Therefore, the quantitative SNIFTIRS analysis has only been performed on the spectral data obtained in deuterium oxide to provide a more accurate measurement of the IR intensity of the pyrimidine ring vibration by ensuring that this IR band is free from all spectral interferences.

Quantitative SNIFTIRS of adenine adsorbed on Au(111) electrode.

The p-polarized SNIFTIR spectra of the Au(111) electrode in a 0.5 M NaF/D₂O electrolyte solution containing 2 mM of adenine at given sample potentials (E_{ads}) are presented in Figure 2. The negative-going component of the IR band at 1640 cm⁻¹ is enhanced and shifted to slightly higher wavenumbers compared to the positive-going portion of the band. The SNIFTIR spectrum obtained using s-polarized photons at a sample potential of 0.09 V is also included in Figure 2. This spectrum contains only the positive band at 1639 cm⁻¹ due to a decrease in the concentration of the desorbed adenine molecules within the thin layer cavity. A comparison of this s-polarized band and the transmission IR spectrum with the positive part of the bipolar band in the SNIFTIRS spectrum recorded using p-polarized radiation at the same sample potential

confirms that the positive lobe of the bipolar band does in fact correspond to the absorbance by desorbed molecules.

- Figure 2 –

The high signal-to-noise ratio of the intense negative band makes it ideal for accurately calculating the angle between its transition dipole and the normal direction to the electrode. The direction of the transition dipole of this vibration is nearly parallel to C4-C5 bond direction. However, two different transition dipole angles are needed in order to determine the orientation of the molecule plane relative to the electrode surface. For this purpose, the band appearing in the SNIFTIR spectra at 1380 cm^{-1} was also selected for further quantitative analysis. According to DFT calculations, this IR band is assigned to the ring stretching vibrations (mainly C5-N7) and the in-plane CH bending mode. This vibration involves a transition dipole moment that is oriented in a different direction (approximately the same direction of the C5-N7 bond) and provides sufficient intensity to allow for quantitative analysis. According to the DFT calculations, the angle between the directions of transition dipoles of the bands at 1640 cm^{-1} and at 1380 cm^{-1} is equal to 85 ± 5 degrees.

To obtain the orientation of adsorbed molecules, the IR contribution of the species desorbed in the solution must be removed from the experimental p-polarized SNFTIR spectrum using the following procedure. First, the s-polarized SNIFTIR spectra are recorded at each sample potential. However, the average mean square electric field strengths of the s-polarized, $\langle E_s^2 \rangle$, and p-polarized $\langle E_p^2 \rangle$, radiation within the thin layer cavity are not equal. Therefore, the magnitude of the electric field strength of the

experimental s-polarized SNIFTIR spectrum, $\left(\frac{\Delta R}{R_{des}}\right)_{s(\text{exp})}$, must be normalized with that

of the p-polarized spectrum using the following correction:

$$\left(\frac{\Delta R}{R_{des}}\right)_{s(\text{norm})} = \left(\frac{\Delta R}{R_{des}}\right)_{s(\text{exp})} \times \frac{\langle E_p^2 \rangle}{\langle E_s^2 \rangle} \quad (4)$$

The average mean square electric field strengths within the thin layer cavity of the spectroelectrochemical cell can be evaluated on the basis of Fresnel equations provided that the angle incidence and thickness of the electrolyte are known.^{18, 34, 37}

These two parameters can be determined using the procedure described in reference¹⁸. The normalized s-polarized SNIFTIR spectra can then be used to remove the IR contribution of the desorbed adenine molecules from the p-polarized SNIFTIR spectra to give the IR absorption spectra of the adsorbed adenine molecules:

$$\left(\frac{\Delta R}{R_{des}}\right)_{p(\text{ads})} = \left(\frac{\Delta R}{R_{des}}\right)_{p(\text{exp})} - \left(\frac{\Delta R}{R_{des}}\right)_{s(\text{norm})} \quad (5)$$

An example of this correction procedure is presented in Figure 3a) for both the 1380 and 1640 cm^{-1} spectral bands at a potential of -0.11 V vs. Ag/.

-Figure 3

As previously mentioned, the quantitative SNIFTIRS analysis is performed by calculating the ratio of the integrated areas of a vibrational band in the experimental spectrum of adsorbed molecules and the theoretical spectrum of a film of randomly oriented molecules of the same surface concentration according to equation (2):

$$\frac{\int A_{p_{\text{ads}}} d\bar{\nu}}{\int A_{p_{\text{calc}}} d\bar{\nu}} = \frac{\int \left(\frac{\Delta R}{R_{des}}\right)_{p_{\text{ads}}} d\bar{\nu}}{\int \left(\frac{\Delta R}{R_{des}}\right)_{p_{\text{calc}}} d\bar{\nu}} = \frac{\cos^2 \theta}{\cos^2 55^\circ} \quad (6)$$

An example of the theoretical p-polarized spectrum is shown in Figure 3b). To calculate the area of the theoretical IR absorption band of a film of randomly oriented molecules, the surface excesses of the adsorbate as a function of the potential must be obtained from thermodynamic measurements. This data has been previously obtained in the thermodynamic study of the adsorption/desorption process of adenine on Au(111) electrodes from neutral solutions.⁷

Additionally, isotropic optical constants, which consist of the refractive index (n_{D3Ad}) and attenuation coefficient (k_{D3Ad}), of deuterated adenine molecules are required to calculate the simulated spectrum at the frequencies of the selected IR bands. These parameters can be obtained from the IR transmission spectrum of deuterated adenine by an iterative procedure described in a previous work.¹⁸ The optical constants of the 1380 and 1640 cm^{-1} vibrations of deuterated adenine, obtained from the IR transmission spectrum presented in Figure 1a), are shown in Figure 4.

- Figure 4-

The adenine optical constants are used to generate the IR absorption spectrum of an adenine film with randomly oriented molecules at the maximum surface concentration, Γ_{max} , at 0.2 V. At all other surface concentrations (Γ), the simulated spectra can be obtained applying a factor of $\Gamma/\Gamma_{\text{max}}$.

The integrated absorbance intensities of the 1380 and 1640 cm^{-1} vibrational bands from both the simulated and experimental spectrum were used to calculate the angles between the normal direction to the electrode and the transition dipoles of the 1640

cm^{-1} (θ_{1640}) and 1380 cm^{-1} (θ_{1380}) vibrations according to Equation (6). The calculated transition dipole angles are plotted as a function of the electrode potential in Figure 5.

- Figure 5 -

Figure 5 shows that θ_{1640} increases from 40° to a limiting value of 52° at a potential of 0.2 V . This suggests that the transition dipole of the 1640 cm^{-1} vibration adopts an orientation that becomes more parallel with the gold electrode surface. In contrast, θ_{1380} decreases from an initial angle of 72° to a limiting value of 63° as the electrode potential becomes more positive. Therefore, the transition dipole of the 1380 cm^{-1} vibration becomes more normal to the electrode surface at more positive potentials.

The angle $\theta_{\bar{\nu}}$ between the transition dipole moment of an in-plane vibration and the normal direction to the electrode includes contributions from two individual angles: i) the angle between the in-plane transition dipole with respect to the projected component of the surface normal of the molecular plane for the in plane vibration, $\varphi_{\bar{\nu}}$ and ii) the angle between the normal of the electrode surface and the molecular plane, which is referred to as the molecular tilt angle α , and is common for all in plane vibrations. The decomposition of the individual angles associated with an adsorbed adenine molecule is presented in Scheme 1.

- Scheme 1 -

From the geometric reasoning provided in the supporting information, it can be deduced that:

$$\cos \theta_{1640} = \cos \alpha \times \cos \varphi_{1640} \quad (7)$$

$$\cos \theta_{1380} = \cos \alpha \times \cos \varphi_{1380}$$

With the aid of these equations and by taking the sum $\varphi_{1640} + \varphi_{1380} = 85 \pm 5^\circ$, the values of the tilt angle of the molecular plane, α , and the rotation angles of the transition dipoles of the absorption bands at 1640 cm^{-1} and 1380 cm^{-1} , φ_{1640} and φ_{1380} , can be calculated. These values are plotted against the electrode potential in Figure 6.

- Figure 6 -

The tilt angle α of the adsorbed adenine molecular plane in Figure 6a is nearly independent of the applied electrode potential with an average value of $\sim 40^\circ$. This inclination of the molecular plane is in good agreement with the conclusions of the thermodynamic study that suggested a tilted orientation of the molecular plane.⁷ It is also consistent with the structure observed in the electrochemical STM images of adenine adlayers on Au(111) electrodes,¹³ where the reported distance between the adenine molecules was found to be between 3 to 4 angstroms. This distance is too short to correspond to intermolecular hydrogen bonding or van der Waals interactions, but does fall within the expected distances for parallel π -stacking interactions. The inclination of molecular planes found from the SNIFTIRS analysis indicates that this π - π interactions must be of the “parallel displaced” type,^{38, 39} instead of the “parallel-sandwich” type. Assuming that the distance between adjacent adsorbed molecules at the surface is of 3.5 \AA with a slope of 40° , the effective displacement is only 2.3 \AA . In the case of solid adenine, crystallographic structure measurements¹⁴ concluded that the molecules are stacked parallel-displaced with the non-overlapping dipoles as a consequence of a twisting film structure. The tilt angle of the adenine adsorbed molecules can be facilitated by the sp^3 hybridization of the

amine nitrogen N10.⁷ This hybridization was also predicted by other authors,^{40, 41} who studied the coordination of adenine to metals on the basis of ab-initio calculations and suggest that the sp³ hybridization of the N10 nitrogen facilitates the adsorption to the metal.

In Figure 6b plots the values of φ_{1640} and φ_{1380} as a function of the electrode potential. The data suggests that as the potential increases from -0.3 to 0.2 V vs. Ag/AgCl, φ_{1640} increases from 18° to a limiting value of 34° and φ_{1380} decreases from 66° to 50°. These changes are caused by a 16° rotation of the molecular plane around its normal axis. This rotation is modeled in Scheme 2 and considers that the adsorbed adenine can interact with the gold surface via the amine nitrogen (N10) and imidazole nitrogen (N7) according to Scheme 2a, or alternatively via the N10 and N1 nitrogen atoms as shown in, Scheme 2b. Both of these models have been previously postulated by other research groups.^{40, 41, 42, 43, 44, 45, 46, 47, 48}

- Scheme 2 -

In the case of the interactions in Scheme 2a, the changes in the transition dipole angles with increasing electrode potential implies a counterclockwise rotation of the molecular plane around its normal direction. This rotation cannot be caused by the interaction between the permanent dipole of adenine, which has a magnitude 2.37 D and directed close to the C5-N7 bond direction, and the electric field at the interface, because the rotation decreases the magnitude of the dipole moment component in the direction of the permanent field. On the other hand, if the interactions between adenine and the metal involve the N10 and N1 atoms (shown in Scheme 2b) then changes in the φ_{1640} and φ_{1380} angles would involve a clockwise rotation in the

molecular plane as the potential increases, the component of the molecular permanent dipole moment would increase in the direction of the electric field. Therefore, proposed orientation in Scheme 2b is more consistent with the expected dipole-field interaction.

The proposed coordination of adenine to the metal through N10 and the N1 atoms also provide an explanation for the pH dependence that was previously observed.¹¹ The adsorbing adenine molecule, from either acidic or neutral solutions, adopts the deprotonated structure, even at pH values below pKa regardless of the fact that the adenine in the bulk of solution consist solely of adenine molecules where the N1 atom is protonated. Therefore, the adsorption mechanism must involve the deprotonation of the N1 site in order to chemically interact with the metal surface.

A more quantitative picture of the evolution of the electrostatic interactions of adenine with the electric field can be obtained by evaluating the component of the molecular dipole moment in the electric field direction, μ_z , as a function of the charge density of the electrode charge. The values of this component are related to the $\cos^2 \theta_z$ values, with θ_z being the angle between the permanent dipole of the molecule, μ_o , and the static electric field direction. The values of $\cos^2 \theta_z$ have been obtained from α and either φ_{1640} or φ_{1380} and are plotted in Figure 7 as a function of the inverse of the surface charge density, σ_M , on the metal. The observed trend can be approximated by a linear decrease, which is expected for electrostatic interactions according to the Langevin model.⁴⁹ For a freely rotating polar molecule in the presence of a static electric field, the Langevin-Debye equation predicts a linear decrease of $\cos^2 \theta_z$ with reciprocal of σ_M :

$$\cos^2 \theta_z = \left(1 - \frac{2\varepsilon k_B T}{\mu_o \sigma_M}\right) \quad (8)$$

where ε is the dielectric constant and $k_B T$ is the thermal energy.

- Figure 7 –

In Figure 7, a linear relationship is observed with a slope of ca. $-4.6 \mu\text{C cm}^{-2}$. Evidently, this value cannot be explained by eq. (7) if adopting the value of the electric permittivity of bulk water. This is not surprising as it is well known that organized water at the electrochemical interfaces adopts a much lower value (see, for instance reference ⁵⁰). Moreover, in the case of adsorbed adenine monolayer the potential drop takes place mostly across an ordered film of an organic dielectric. If the square of adenine refractive index, n_{D3Ad} in Figure 4, is adopted for the dielectric constant in this case the value of the slope must be in the range -0.8 to $-1.5 \mu\text{C cm}^{-2}$, which is of the same order of magnitude than the experimental slope value.

CONCLUSIONS.

A quantitative SNIFTIRS procedure has provided physical calculations of the angles between the normal direction to the electrode and the transition dipoles of two selected in-plane vibrations (θ_{1640} and θ_{1380}) of adenine adsorbed on Au(111) electrodes. Using these values, the tilt angle, α , of the molecular plane with respect to the electrode surface, and the two angles between each of the two selected transition dipoles and the projected normal direction to the electrode on the molecular plane, φ_{1640} and φ_{1380} , have been evaluated at different potentials within the chemical

adsorption potential range. The dependence of φ_{1640} , φ_{1380} and α angles on the applied potential was determined.

The value of α is $\sim 40^\circ$ at potentials higher than the onset of adenine adsorption, in agreement with a sp^3 hybridization of the N10 atom. This hybridization facilitates its coordination to the electrode, and suggests that the stabilization of the adsorbed monolayer is due to π - π parallel displaced stacking interactions between adjacent adsorbed molecules.

The changes with potential of the φ_{1640} and φ_{1380} angles of the two selected transition dipoles were used to determine rotation of the molecular plane around its normal axis. These changes are consistent with coordination of the adenine molecule through the N10 and the N1 atoms with the metal surface. Thus, it is concluded that adenine adsorbs on the gold electrode with the N10 and N1 atoms directed towards the electrode surface and its molecular plane increasingly rotates over a range of about 16° when increasing the static electric field intensity. This molecular organization provides an explanation about why the neutral deprotonated form of adenine is adsorbed at the gold surface even from solutions of very low pH values.

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LEGENDS

Scheme 1.- Representation of an adsorbed adenine molecule on the Au(111) electrode with a tilt angle of the molecular plane, α , relative to the normal direction of the electrode surface. φ_{1640} and φ_{1380} are the angles between the corresponding transition dipoles vibrations and the projected normal to the electrode on the molecular plane.

Scheme 2.- Representation of the adenine molecular plane adsorbed on Au(111) electrodes. The projected normal direction to the electrode on the molecular plane (black dashed line), the approximate directions of the transition dipoles of the vibrations at 1380 (green dashed line) and 1640 cm^{-1} (red dashed line), respectively. The blue arrow represents the permanent molecular dipole direction. The interaction of the adenine molecule with the metal surface via the a) N10 and N7 atoms or b) the N10 and N1 atoms.

Figure 1.-Transmission absorption spectrum of 10 mM adenine in a) 0.5 M NaF/D₂O and b) 0.5 M NaF/H₂O.

Figure 2.- The p-polarized SNIFTIR spectra of deuterated adenine adsorbed on Au(111) electrodes in 2mM solutions in 0.5 M NaF/D₂O at selected sample potentials with a reference potential of -0.65 V vs. Ag/AgCl. The s-polarized SNIFTIR spectrum at 0.05 V vs Ag/AgCl is included for the comparison.

Figure 3.- a) The experimental p-polarized (blue dashed line) and s-polarized (red solid line) SNIFTIR spectra around the bands at 1380 and 1640 cm^{-1} of deuterated adenine adsorbed on Au(111) electrodes from 2 mM solutions in 0.5 M NaF/D₂O at -0.11 V vs.

Ag/AgCl and the corrected p-polarized SNIFTIR spectrum according to Equation (3) (red solid line). b) The simulated SNIFTIRS bands at 1380 and 1640 cm^{-1} for an adenine film consisting of randomly oriented molecules with a surface concentration of 2.2×10^{-10} mol cm^{-2} .

Figure 4.- a) Refractive index and b) attenuation coefficient of deuterated adenine for the 1640 and 1380 cm^{-1} wavenumber regions.

Figure 5.- Potential dependence of the transition dipole angles of the 1640 cm^{-1} (●) and 1380 cm^{-1} (Δ) vibrations relative to the normal direction of the electrode surface for an adsorbed deuterated adenine film. The schematic representations of the transition dipole directions in the molecular plane are included.

Figure 6- .- a) Angle of the molecular plane of adsorbed adenine as a function of the potential. b) The potential dependence of the angles relative to the projected normal direction of the electrode within the molecular plane of the transition dipoles moments of the 1640 cm^{-1} (●) and 1380 cm^{-1} (Δ) vibrations of deuterated adenine adsorbed on Au(111). The error bars account for 5 degrees of uncertainty in the calculation of the angle between the transition dipoles of the vibrations at 1640 and 1380 cm^{-1} .

Figure 7.-The cosine square of the angle between the permanent dipole moment of an adsorbed adenine film and the static electric field with respect to the inverse of the charge density on the metal surface .