CHAPTER 4:

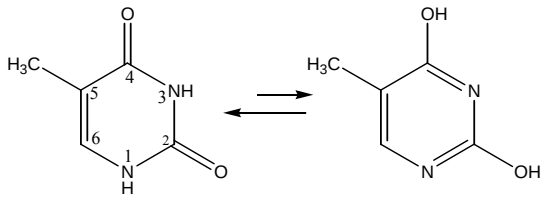
ADSORPTION OF THYMINE / ADENINE ON AU(111) ELECTRODE

In today's age of molecular biology purines and pyrimidines are probably best known as the basic constituents of the polynucleotides DNA and RNA. Of predominant importance are the ribo- or deoxyribonucleotide forms of the five purine and pyrimidine bases; two purine bases (adenine and guanine) and three pyrimidine bases (cytosine, uracil and thymine). Adenine and guanine consist of a six-membered and a five-membered nitrogen-containing ring, fused together occurring in both DNA and RNA. Pyrimidines have only a six-membered nitrogen-containing ring. Cytosine and thymine are the pair of pyrimidines in DNA, and cytosine and uracil are the pair in RNA. Biological importance of purine and pyrimidine bases is not restricted to be constituents nucleic acids. These substances as corrosion inhibitors and electroplating brighteners are also important for some industrial applications.

4.1. Thymine

4.1.1. Chemical Properties of Thymine

Thymine is a pyrimidine base and as such one member of the base pair A-T (adenine-thymine) in DNA. The pyrimidines all display tautomerism. At neutral pH the keto form predominates, but at alkaline pH shifts to the enol form.



Keto Enol

Figure 4.1: *Tautomeric forms for thymine.*

At alkaline pH the hydrogen N(3) for thymine is removed, indicating the weak basicity of the ring nitrogen.

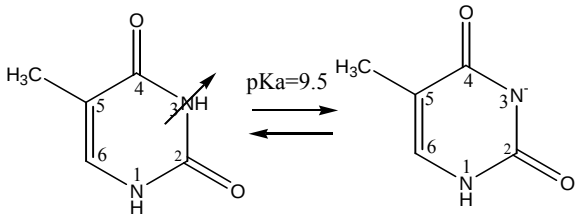


Figure 4.2: *Ionization constant for thymine. Arrow indicates the dipole moment.*

4.1.2. Adsorption of thymine on Au(111)

The adsorption of thymine has been investigated by electrochemical methods on surfaces such as on Hg [13, 55], Au(111) [14, 106] and Ag(111) [107] electrodes. Measurement techniques used in such studies include electrochemical methods such as voltammetry [25-27, 53], capacitive measurement [14, 27, 108], and chronocoulometry [108], while more recently modern electrochemical methods such as in-situ STM [14, 26, 27], SERS, IR spectroscopy [26] and XPS [14, 27, 53] have been applied.

It is well known that the DNA bases undergo a two-dimensional first-order phase transition on mercury and single crystal electrodes, forming well-ordered condensed monolayer [19-22]. The phase transition process depends on parameters such as bulk concentration, temperature and electrode potential [30].

The cyclic voltammogram and the capacitance curve of 12 mM thymine on Au(111) in the electrolyte containing 0.1 M $HClO_4$ and 0.060 M $NaClO_4$ is shown in Fig. 4.3. These results are in good agreement with most of publications. Depending on the potential two different, well-ordered adsorption layers (physisorbed and chemisorbed) at four potential regions are identified for the adsorption of thymine on Au(111). The critical potential where the layers change is around the potential of zero charge (PZC) located at -0.22 V.

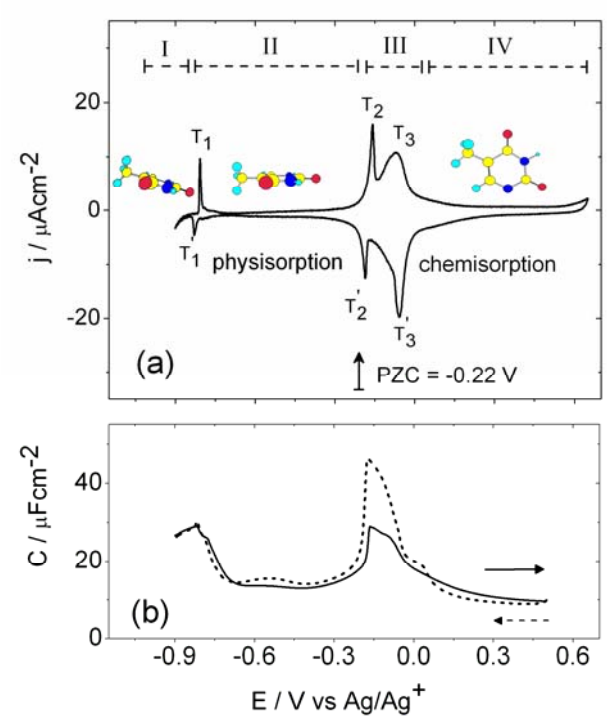


Figure 4.3 a: *The cyclic voltammogram of 12 mM Thymine + 0.1 M $HClO_4$ + 0.060 M $NaClO_4$ on Au(111). Sweep rate: 50 mV/s.* ***b:*** *The corresponding capacity-potential curve. Perturbation frequency and amplitude: 80 Hz, and 3 mV, respectively.*

Negative of PZC: At the negative end of the potential scale (*region I*) a disordered phase is formed, which is followed by a two-dimensional phase transition (T_1, T_1') into a so-called physisorbed condensed phase (*region II*). The plane of thymine molecules is oriented nearly parallel to the electrode surface as was confirmed by STM [14, 26, 27] and SNIFTIRS [26] experiments. Voltammetric data indicate that the formation of the phase in region II is pH-independent [109] and relatively temperature-sensitive [12], indicating that the thymine adsorbate exists in their un-deprotonated form and the adsorption phase is weak (hence donated "physisorbed"). This physisorbed phase has been previously imaged by in-situ STM which indicates planar-oriented molecules in a hydrogen-bonding network [14, 27]. In the state of random adsorption molecules are slightly tilted with one of its nitrogen bonds towards the surface [26].

Positive of PZC: *At potential region III* a pair of pronounced current peaks is observed (T_2, T_2' and T_3, T_3') whereby the physisorbed monolayer is transformed into a (condensed) chemisorbed adsorption state of thymine at positive potentials (*region IV*). The formation of

chemisorbed phase is a complex process (*region III*) which takes place simultaneously with the lifting of the reconstruction of the surface. Thymine molecules form a two dimensional phase showing high stability to temperature changes [12]. The transition to the chemisorbed phase is quite different, with a large current flow in region III, resulting in the formation of a chemically modified adlayer ("chemisorbed") in region IV. Thymine molecules form stacks in this state with water molecules [27, 30]. STM imaging of the chemisorption phase of thymine indicates the upright orientation of the standing molecules stacking in rows on the surface [14, 25]. Hölzle et al. [14] showed that the electrochemical behavior of several methylated derivatives of uracil, including thymine, shares a similar adsorption behavior and proposed an adsorption model for the chemisorbed phase of these molecules. In this model the molecule is deprotonated and chemically bound to the metal surface through one of the nitrogen ring atoms.

Following the same line, Haiss et al. [26] studied the adsorption of thymine on Au(111) with SNIFTIRS study to probe the structure and bonding of the 'chemisorbed' thymine phase, and found that thymine indeed chemisorbs to a gold surface through a nitrogen atom. This layer would be bound to the metallic surface (gold or silver) through one of the nitrogen atoms, probably N(3) in the numbering scheme illustrated in Fig. 4.4.

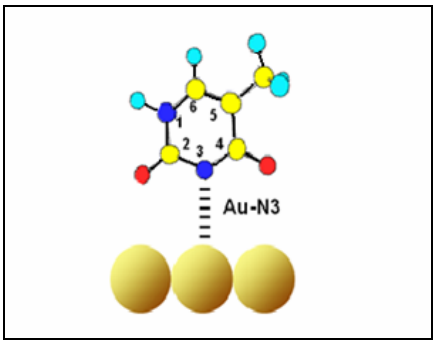


Figure 4.4 *The structural representation of chemisorbed thymine on Au(111) via N(3).*

The typical single frequency (80 Hz) capacitance vs. potential curves for 12 mM Thymine in 0.1 M $HClO_4$ on Au(111) are plotted in Fig. 4.3.b. The experiment started from both ends of potential scale after immersion without potential control. The positive as well as negative going scans are indicated by dashed and solid lines, respectively.

Four characteristic potential regions could be distinguished. They can be labeled like the voltammogram. Region I, at E < -0.75 V exhibits a high capacitance and the transition between region (I) and region (II) is characterized by a relatively sharp capacity peak which indicates the increase of the surface coverage of adsorbed thymine from state I to state II. In potential regions II and IV exhibit low capacitance and pronounced hysteresis. The saturation capacitances of about 13 and 9 μFcm^{-2} are established only in region II and region IV, respectively. The transition between the states II and V is also characterized by a capacitance peak, whereby the height of the peak depends on the direction of transition. This behavior shows us that the transition from physisorbed into the chemisorbed state is significantly slower than the reverse process. In the region (IV) the capacitance reaches a low and constant value; lower than the second region, which means that the thymine molecules are strongly adsorbed on the electrode surface.

4.1.2.a Concentration Dependence

The concentration dependence of thymine adsorption on the Au(111) electrode at 20 °C are illustrated in Fig. 4.5.a-c corresponding to 0.5, 1 and 12 mM, respectively (in 0.1 M $HClO_4$ + 0.060 M $NaClO_4$). It is obvious that the peak pairs $T_1/T_1`$ and $T_2/T_2`$ show a dependence to the concentration changes. The sharp and intense $T_1/T_1`$ peak pair observed in 12 mM thymine gets smaller and shifts ca. 0.3 V to positive potentials in 1 mM thymine. Observing the small peak pair observed in 1 mM thymine solution depends on the quality of the electrode. In case of 0.5 mM of thymine, this peak pair almost disappears. The intensity of $T_2/T_2`$ peak pair decreases with lowering concentration and disappears at 0.5 mM concentration of thymine, but the position of it does not depend on the concentration change. It can be concluded that the potential window at which hydrogen bonded network formation of thymine molecules takes place gets narrower with lowering concentration and at lower concentrations ($c \leq 1$ mM) thymine molecules cannot form a networked structure.

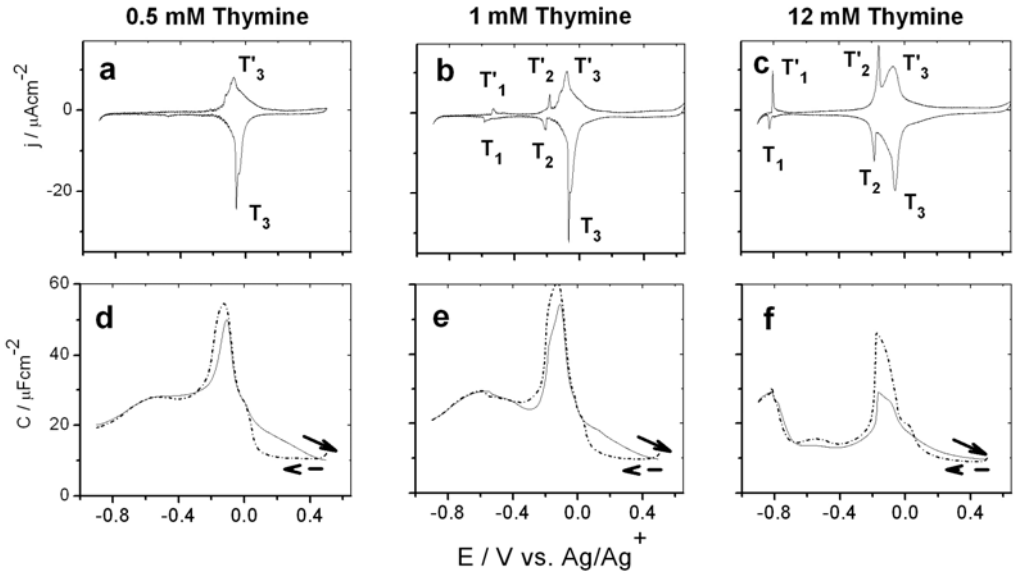


Figure 4.5 *The cyclic voltammograms and capacity-potential curves of 12 mM (a,d), 1 mM (b,e) and 0.5 mM (c,e) Thymine on Au(111) in 0.1 M $HClO_4$ + 0.060 M $NaClO_4$. Sweep rates: 50 mV/s. Perturbation frequency and amplitude: 80 Hz, and 3 mV, respectively.*

The capacitance measurements (Fig. 4.5.d-f) carried out at 80 Hz frequency also indicate that the physisorbed state of thymine adsorption obviously depends on concentration changes. The potential range, where the physisorbed state has a minimum capacitance (-0.7 V - -0.2 V), becomes shorter with lowering concentration. The capacitance curve (Fig. 4.5.f) of 12 mM thymine solution possess a well-defined minimum pit region (between -0.700 V and -0.250 V) indicating the formation of an intense hydrogen bonded network of thymine molecules on the gold surface. At positive potentials (region IV), the capacitance value has its minimum for all measurements indicating the presence of upright oriented chemisorbed phase of thymine.

4.1.2.b Acidity Dependence

We have investigated the adsorption behaviour of thymine on Au(111) depending on the acidity of the electrolyte. The following cyclic voltammograms (Fig 4.6) of 12 mM thymine electrolytes containing different perchloric acid concentrations [10^{-1} M (a), 10^{-3} M (b), neutral (c)] were obtained scanning at 50 mV/s rate and keeping the ionic strength constant of 0.160 M (in $NaClO_4$ electrolyte).

The peak pair, $T_1/T_1`$, where the transition from randomly adsorbed phase to uniformly phsisorbed phase takes place, shifts ca. 50 mV more positive potential as the acid concentration drops from 10^{-1} M (a), 10^{-3} M (b). However, with further lowering the acidity, the position of the peak pair does not change. The peak pairs, $T_2/T_2`$and $T_3/T_3`$, indicating the transition between physisorbed and chemisorbed states, reflects a continuous negative shift with lowering acidity. The results are in good agreement with the findings of Meyer [109].

Summarizing, the potential range of ordered physisorbed phase gets narrower as the acidity decrease. The widths of potential ranges, in which randomly and uniformly physisorption take place, differ reversely; as the region I gets broader, the region II gets shorter with lowering acidity. The whole potential window shifts to negative potentials due the lowering of hydrogen ion concentration.

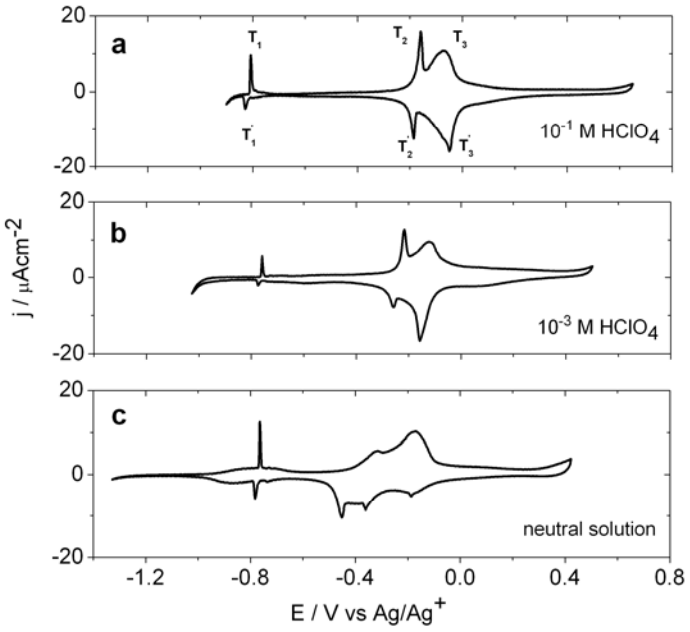


Figure 4.6 Cyclic voltammetries of Au(111) measured in 12 mM thymine with varying acidity of electrolyte (ionic strength is constant, 0.160 M).

Roelfs and Baumgärtel [14] demonstrated for the first time that the transition potential ($T_2/T_2`$) from physisorbed to chemisorbed phase (II to III) depends linearly on the pH in the range between 4 and 10 (-72 $\pm$ 5 mV/pH). However, at lower pH values, the linear dependence is not observed and the transition potential becomes constant. Our findings (with Meyer [109]) at lower pH range is not in close agreement with it. It is clearly observed (Fig. 4.6.a-b) that even at

lower pH, the negative shift of (T_2/T_2`) is present with a lower rate of -28 $\pm$ 5 mV/pH. This result means that the idea explaining the behaviour of thymine on the gold surface is valid at lower pH value, too.

Their explanation for negative changing when increasing the pH was based on the higher mobility of the protons in solution compared to the perchlorate anions. The positive/negative potentials lead to an accumulation of anions/cations near to the electrode surface, in case of medium pH. Lowering the pH, even positive potentials are counterbalanced by elimination of protons rather than by accumulation of anions leading to an increase of the pH in the double layer compared to the bulk. In the frame of this explanation, it is proposed that during chemisorption, deprotonation of the thymine molecule followed by a partial discharge takes place. The formed monoanions exists as a mixture of the N(1) and/or N(3) deprotonated forms being able to bind to the electrode surface. The following scheme (Fig. 4.7) of Roelfs *et al.* [14] associated to the deprotonation explains not only the pH dependence and the charge due to formation of anionic species, but also the high stability of the chemisorbed film.

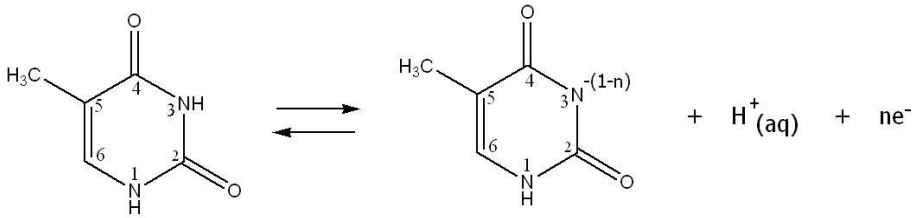


Figure 4.7: *Deprotonation of chemisorbed thymine on Au(111)* [14].

Additionally, XPS [14, 110, 111] and SNIFTIRS [26] experiments strongly suggested that thymine and gold surface atoms interact via N(3) rather than N(1).

4.2 Adenine

Adenine is one of the most important organic molecules for life and an integral part of DNA, RNA, and ATP. It is through the precise inheritance of on organism's DNA from its parent that the traits of an organism are passed on. Besides DNA and RNA, adenine is also an important part of adenosine triphosphate, or ATP. Adenosine triphosphate is the nitrogenous base adenine bonded to a five carbon sugar. This molecule is important because it has the ability to

phosphorylize, or adds a phosphate group to other molecules. This transfer of a phosphate group allows energy to be released. It is this energy which is used by cells in living organisms. This is why the molecules ATP, and its nitrogenous base Adenine, are so important. Adenine also occurs as a component of a number of coenzymes, for example coenzyme I: is nicotinamide adenine dinucleotide (NAD^+) or diphosphopyridine nucleotide (DPN^+), coenzyme II is nicotinamide adenine dinucleotide phosphate ($NADP^+$) or triphosphopyridine nucleotide (TPN^+). Coenzymes of these types are often involved in oxidation-reduction process in biological reactions together with the appropriated enzyme.

4.2.1 Chemical Properties of Adenine

The tautomeric forms of adenine are the *amine* and *imino* forms (Fig. 4.8), respectively.

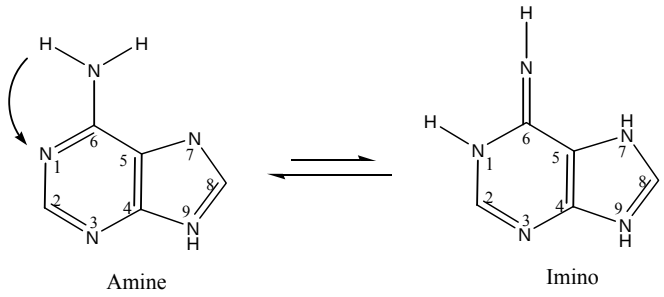


Figure 4.8: *Tautomeric equilibrium of adenine.*

Although adenine can undergo a change from an amine to an imino form, the amine form is strongly favored being more difficult to detect even traces of the imino form in the naturally occurring DNA. However, if the less stable form is present in DNA it can seriously alter the DNA sequence by forming base pairs other than the standard ones. Normally, adenine and thymine form the AT standard pairs, however the imino form from adenine can form a stable hydrogen bonded pair with cytosine, AC instead of AT, causing a mutation process.

In acidic media, adenine undergoes protonation on the N1 position rather than on the amino group. The charged form is stabilized by the resonance hybrids. Crystallographic studies have shown that at pH < 2 adenine is double protonated at N1 and N7 nitrogens and the bonding geometry is changed according to the resonance structures (Figure 4.9). As a result of

protonation, π electron localization on the ring(s) increases and hence force constants of the bonds increase.

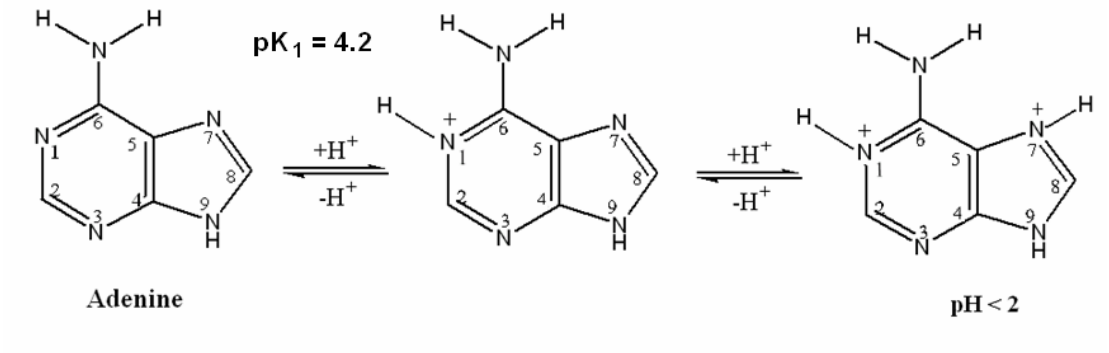


Figure 4.9: *Protonated forms of adenine.*

4.3.2. Adsorption of Adenine on Au(111)

The adsorption and condensation behavior of adenine in different electrolytes on mercury electrodes has been the subject of several papers [57, 112, 113]. Adenine undergoes a first order phase transition depending on thermodynamic parameters such as temperature, potential and concentration. The kinetics of the condensation on mercury has recently been studied by Buess-Hermann et. al [58].

Adsorption behavior of adenine on Au(111) electrode has not been investigated as much as that of on mercury. The present in situ STM and AFM studies of condensed adenine films on solid electrodes have shown the existence of ordered structures, either stacked polymeric chains on Au(111) [114] or hydrogen-bonded planar network on graphite electrodes [59]. Adenine adsorption seems to be very sensitive to the nature of the substrate. On Au(111) electrodes, Donner and Camargo [12, 24] have shown that adenine adsorption do not cause the needle peaks characteristic of two dimensional condensation, as observed in the case of thymine and uracil adsorptions.

Xiao *et al.* has investigated the potential dependent adsorption of nicotinamide adenine dinucleotide (NAD^+) on roughened Au electrode in neutral electrolyte by SERS measurements [60]. Based on the difference in SERS spectra at negative and positive potential region, they concluded, in agreement with the STM studies [114], that under negative potential the adenine moiety of NAD is chemisorbed in a flat orientation forming a charge-transfer complex between

the π^*-orbital (LUMO) of adenine and the d-orbital of Au(111). Formation of the charge transfer complex is proved by the shifting of the bands, compared to the normal spectra in solution. The parallel geometry allows a partial electron transfer from the gold d-orbitals to the π^*-orbital of adenine. A second chemisorbed adsorbate of adenine exists at more positive potentials (in this potential range, thymine is also chemisorbed). In contrast, with positive potentials, the N7 atom and NH_2 group of the adenine are adsorbed in a vertical orientation respect to the surface of the electrode.

In our study, we have investigated the adsorption behavior of adenine (2 mM) on Au(111) in 0.1 M $HClO_4$ and 0.060 M $NaClO_4$. From Fig. 10, one can distinguish four different potential regions in the CV and in the corresponding differential capacity curve.

Region I: The first region at negative potentials shows no significant changes in both CV and the capacitance curve. The average value of the capacitance is 28 ± 2 μFcm^{-2}. In this region, adenine molecules form disordered and planar oriented charge transfer complexes with the surface gold atoms [115].

Region II: In the second region, in the CV a couple of broad A_1/A_2 and A'_1/A'_2 peaks (dissolution and formation of the charge transfer complex, respectively [12, 60]) are observed. Additionally, capacity curve has also very broad peak. According to Martins et. al., in this region, adsorbed adenine molecules undergo a deprotonation with simultaneous lifting of the reconstruction. At the end, an ordered physisorbed film on the non-reconstructed gold surface is formed.

Region III: At the negative side of third region, the capacitance is at the lowest value (16 ± 2 μFcm^{-2}) suggesting the reorientation of adenine to more vertical position compared to that in region 1. In this region, there is no significant change in the voltammogram, whereas the capacitance increases gradually towards positive potentials indicating the change of orientation from vertical to tilted position (capacitance is inversely proportional to the distance between the parallel plates).

Region /V: At the positive end potentials a broad peak pair with a small shoulder corresponds to the oxidation and reduction of gold (A_3/A'_3) in the adenine-gold complex [12], whereby the capacitance increases slightly. This peak pair locates approximately 60 mV negative

of the gold oxidation in pure electrolyte. Camargo [54] proposes that deprotonation of adenine takes place in this region and it leads to a chemisorption process, as observed with thymine.

At very positive potentials where the gold surface atoms were undergone to oxidation, adenine also causes the inhibition of oxidation like thymine, but at lower degree.

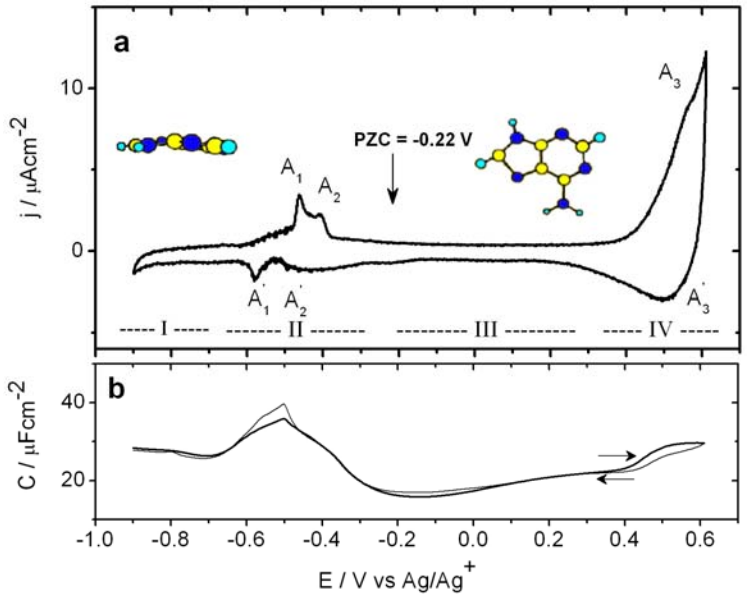


Figure 4.10. a: *The cyclic voltammogram of 2 mM Adenine in 0.1 M $HClO_4$ + 0.060 M $NaClO_4$ electrolyte on Au(111) . Sweep rate: 50 mV/s.* ***b:*** *Capacity curve: perturbation frequency: 80 Hz, amplitude: 3 mV.*

4.3.2.a Acidity Dependence

Figure 4.11 demonstrates the acidity dependence of adenine (2 mM) adsorption in the electrolytes ($HClO_4$, $NaClO_4$) having constant ionic strength (0.160 mM). All peak positions strongly depend on the acidity of the electrolyte. At lower acid concentration, the peak pairs shift to negative potentials. Additionally, the current density for the peaks A_1/A'_1 and A_2/A'_2 increases with decreasing acidity, as observed also by Camargo [54]. This is because, in acidic media, the binding sites of adenine are occupied by protons and tend to be rejected by the positively charged electrode. Therefore, deprotonation in acidic solutions can occur at positive potentials compared to less acidic ones. It should be noticed that adenine is doubly protonated in the solutions having pH lower than 2 from N(1) and N(7) atoms.

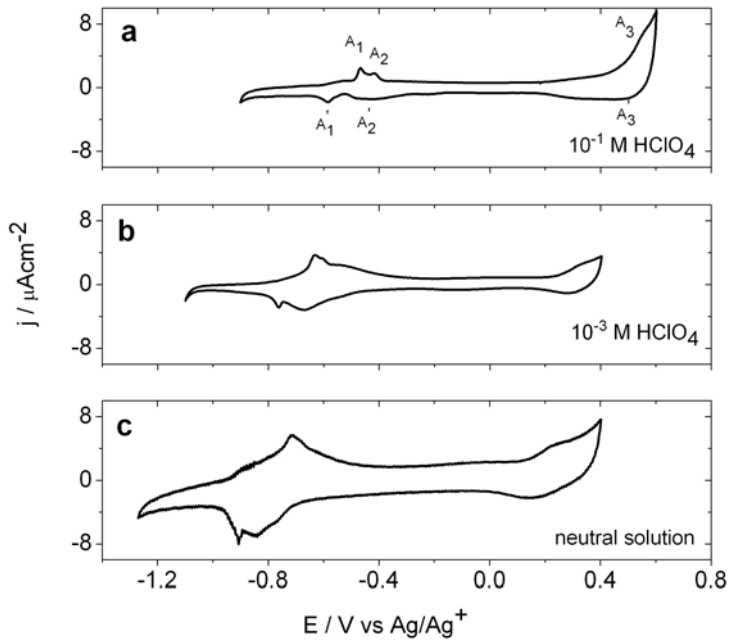


Figure 4.11*. Cyclic voltammetries of Au(111) measured in 2 mM adenine with varying acidity (ionic strength is constant, 0.160 M). Scan rates are 50 mV/s.*

It can be concluded that the deprotonation of (doubly) protonated adenine is less favored in highly acidic electrolytes, so the process is observed at more positive potentials. However, in less acidic solutions adenine, adenine is either mono-protonated (pH>2) or neutral (pH>6). So, deprotonation takes place at negative potentials. The reason behind the larger peaks (A_1-A_1', A_2-A_2') with lower acidity is the increase of the charge transferred, hence the increase of chemisorption level.

4.3.3 Comparison of adenine system with thymine

As it is known from the adsorption behavior on mercury electrodes adenine adsorbs considerably more strongly than thymine. Camargo et. al investigated that this is also valid for the adsorption of adenine on Au(111) [12, 24]. It is proved by comparing the adsorption behavior at different concentration ratios of both bases. They found that for concentration ratios adenine/thymine greater than or equal to 0.2 (pH=2) the obtained CV is identical with that for pure adenine solution.

For a better comparison of both systems, CV (dash) as well as capacitance curves (dash, dot) of 12 mM thymine was also overlaid (dash line) in Fig 4.12.a,b. The most striking feature in

the voltammogram of adenine adsorption is the existence of not so pronounced peaks (A_1,A'_1 and A_2,A'_2), whereas in the voltamogram of thymine all observed peaks are sharper and/or bigger. Such a needle peak pair, T_1,T'_1, indicating the first order phase transition, observed in thymine adsorption, is not seen in the adsorption of adenine. The reorientation of adenine molecules (A_1,A'_1 and A_2,A'_2), takes place at negative of the PZC in contrast to thymine system, whereby it occurs at positive of the PZC.

Despite of the difficulty to determine the onset of gold oxidation in adenine system; thymine significantly inhibits the surface oxidation of gold. However, it should also be considered that the level of oxidation in adenine containing electrolyte is very low compared to the 0.1 M $HClO_4$ electrolyte.

For both systems, the capacitance peaks are located at the potential regions where the phase transition takes place. In thymine system, the capacitive curves exhibit well-defined plateau at the physisorbed and chemisorbed phases. However, in the adenine system, the phases can be hardly distinguished.

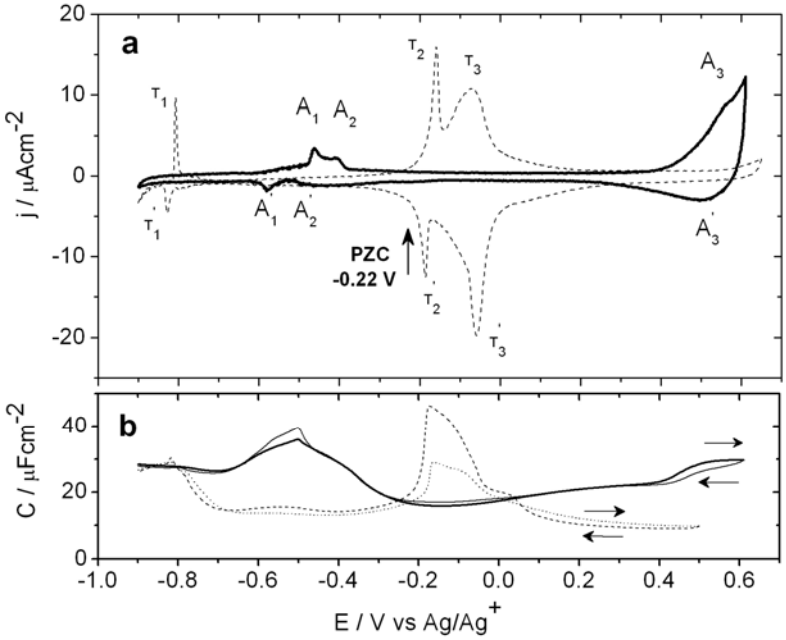


Fig. 4.12.a: *The cyclic voltammograms of 2 mM Adenine (solid) 12 mM Thymine (dash) in 0.1 M $HClO_4$ + 0.060 M $NaClO_4$ electrolyte on Au(111). Sweep rates: 50 mV/s.* ***b:*** *Capacity curves: 2 mM Adenine (solid lines), 12 mM Thymine (dash-dot lines): Perturbation frequency: 80 Hz, amplitude: 3 mV.*

As conclusion, adenine is strongly adsorbed at Au(111) in the double layer region. No first-order phase transitions like in the Au(111)/thymine were observed, but two different states of chemisorbed adenine are evident. At negative potentials the plane of adenine is mainly in a flat orientation with respect to the electrode surface. This geometry allows a partial electron transfer from the Fermi level of the electrode surface to the π^* orbital of the adsorbate.

A second chemisorbed adsorbate of adenine exists at more positive potentials. In this potential range thymine is also chemisorbed. The chemisorbed thymine molecules are oriented perpendicular to the electrode surface. In analogy, we assume a similar orientation for adenine (vertical or tilted) in which the amino groups and N(7) are coordinated to the electrode surface in this potential range.