CHAPTER 6: ADENOSINE AND THYMIDINE

DNA and RNA molecules are linear polymers composed by nucleotides. As already seen in Chapter 1, three parts form nucleotides: a heterocyclic nitrogen base, a sugar and a phosphate group. When the nucleotides are under partial hydrolysis in such a manner that only the phosphate group is lost, the nucleosides are formed. This is schematically given in Figure 6.1.
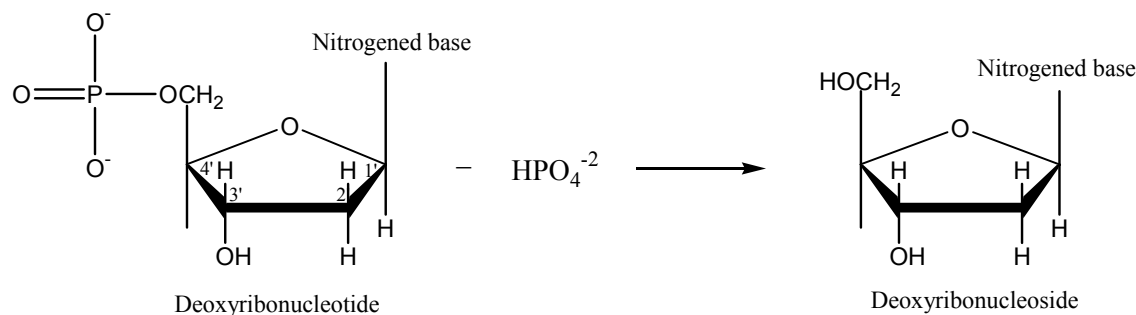

Figure 6.1: Structure of nucleotide and nucleoside.

The purine nucleosides are carbohydrate derivatives of purine in which the purine is linked through N(9) position via the β-n-glycosidic bound to either D-ribose or 2-deoxy-D-ribose. As a result of the two types of sugar moiety associated with the purine in the nucleosides, there are two types of nucleosides: the ribonucleosides and the deoxyribonucleosides. When linked to a ribose (sugar group), adenine corresponding nucleoside is called adenosine and in analogy thymine results thymidine.

Owing to its biological and physiological importance, studies on the physical-chemical properties of adenine nucleotides can be considered of primary importance. The adsorption behavior of nucleosides was firstly studied by Vetterl [60,114] on hanging mercury drop electrode (HMDE) by using alternating current polarograms. In this seminal work the author proposed two different adsorption states for adenosine depending on the potential due to the reorientation of the molecules on the electrode surface.

Although the initial studies where mainly done on Hg electrodes, more recent investigations regarding to the adsorption behavior of adenosine has been reported on different electrode surfaces as Bi(111) and Bi(001) [115], and on glassy carbon electrode (GCE) and pyrolic graphite electrode (PGE) [116], also showing important characteristics

regarding the reaction mechanism. Spectroscopy studies as SERS investigation of NAD$^+$ adsorbed on silver electrode [117] showed a shifting of the spectra when the electrode potentials are changed towards more negative values. According to the differences observed in the intensity of the spectra as a function of the potential, the authors suggested a change of the conformation of the adenosine ribose.

In this chapter are presented the results of the adsorption behavior of the nucleosides adenosine and thymidine, first individually and then when both nucleosides are present in the electrolyte solution. The aim of this chapter is to investigate if and how the sugar group bounded to the nitrogen bases adenine and thymine influences the interaction obtained for the bases only (Chapter 4).

6.1 ADENOSINE

6.1.1 ADSORPTION OF ADENOSINE ON AU(111)

Figure 6.2 shows the cyclic voltammogram of adenosine on Au(111) obtained at temperatures of 20 (a), 10 (b) and 5 °C (c) and the respective capacity curve for T = 5 °C.
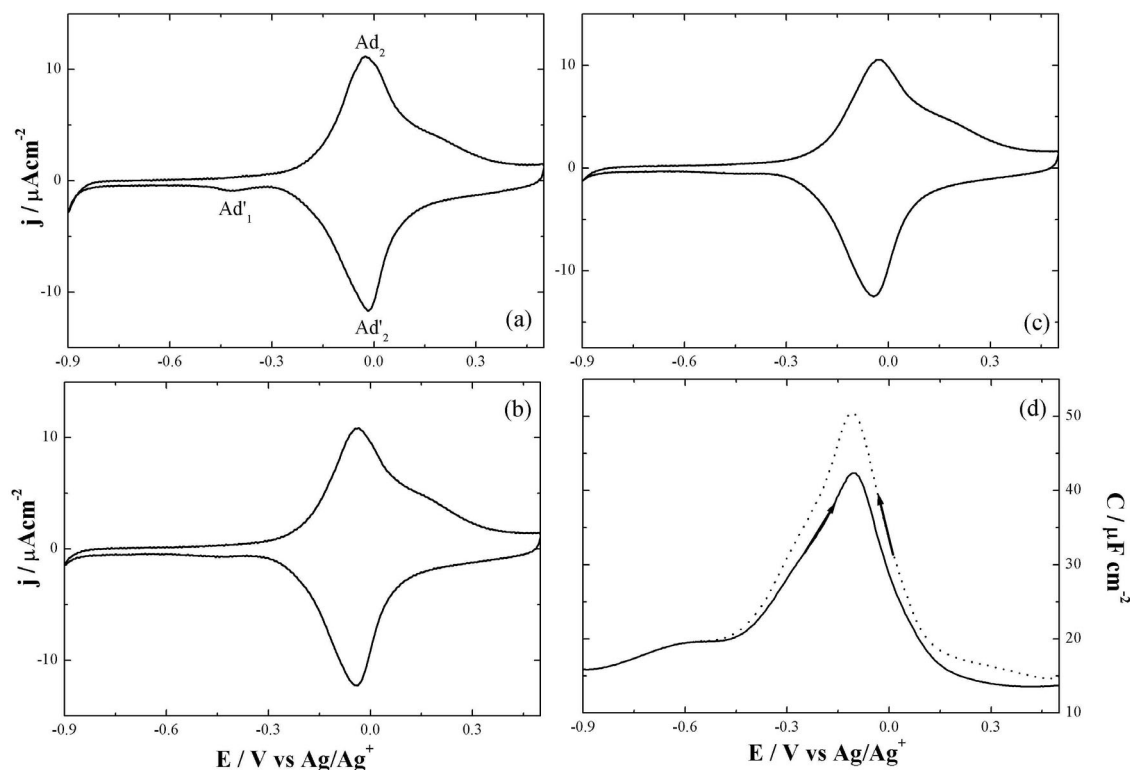
Figure 6.2: Cyclic voltammograms of adenosine 1 mM at T = 20 °C (a), T = 10°C (b) and T = 5 °C (c), and capacity curves at T = 5 °C, (d). Supporting electrolyte: 0.1 M NaClO$_4$ (pH = 2). Sweep rate: v = 50 mV/s (in (a), (b) and (c)) and v = 5 mV/s (d). Perturbation frequency and amplitude when measuring the capacity curve: 100 Hz, and 3 mV, respectively.

The presence of the sugar group at N(9) position on adenine produced a significant change on the CV features when compared with the adsorption behavior of adenine on Au(111) (see Figure 4.19 in Chapter 4,). Comparing the voltammetric profile with adenine base, no discernible current peak was obtained at negative potentials, indicating that the charge-transfer complex postulated for adenine could not be formed. At more positive potentials, a well-defined reduction peak, Ad'$_1$, was only observed at T = 20 °C, disappearing when the temperature was lowered. At potentials around 0 V, a peak pair Ad$_2$/Ad'$_2$ can be seen. The potential of this peak pairs are slightly dependent on the temperature in a range between 20 and 5 °C. On the other hand, the shape of the CVs reminds the adsorption behavior of thymine / uracil. A difference to be stressed when comparing with the thymine adsorption concerns the absence of the phase-transition peaks at negative potentials due to the formation/dissolution of the physisorbed film of thymine.

The effect of the adenosine concentration on the electrochemical response is further investigated in Figure 6.3. These experiments were performed at higher adenosine concentration, namely at 10 mM, at the same pH value than that of the experiments presented in Figure 6.2.
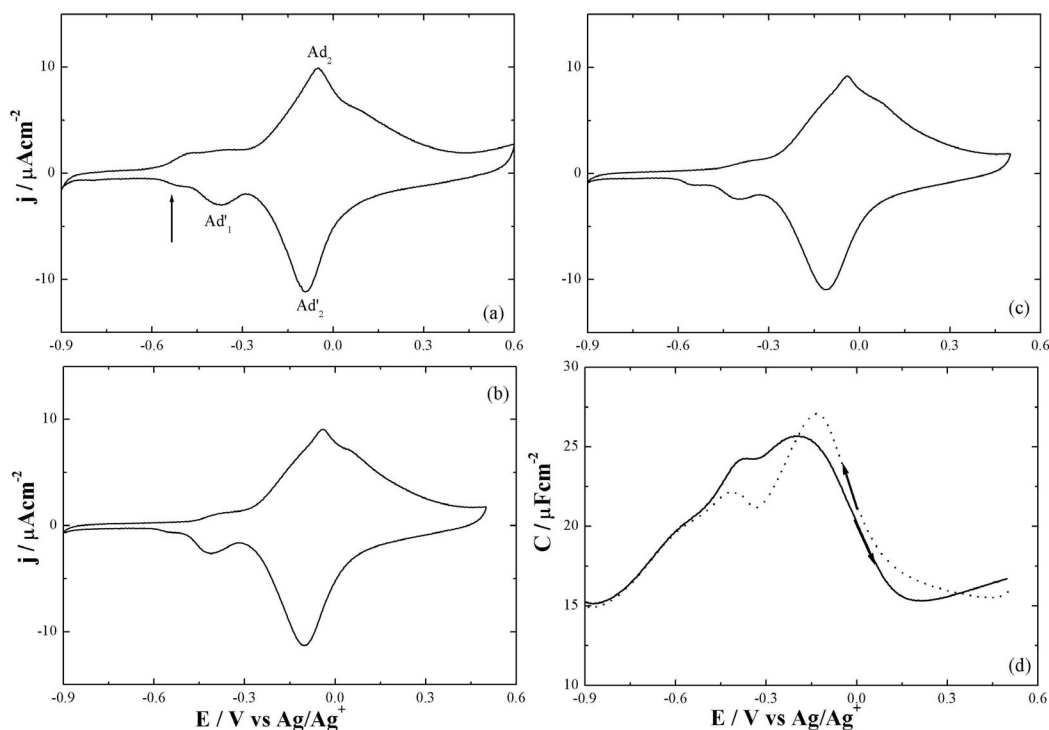

Figure 6.3: Cyclic voltamogramms of adenosine 10mM at T = 20 °C (a), T = 10 °C (b), T = 5 °C (c) and capacity curves at T = 5° C. (d). Supporting electrolyte: 0.1 M NaClO$_4$ (pH = 2). Sweep rate: v = 50 mV/s (in (a), (b) and (c)) and v = 5 mV/s (d). Perturbation frequency and amplitude when measuring the capacity curve: 100 Hz, and 3 mV, respectively.

The first remarkable difference between Figures 6.2 and 6.3 is at the negative potential region. At this range, a new peak (indicated by the arrow) appeared and the current for the peak Ad'$_1$ increases with increasing adenosine concentration, c.f. Figure 6.3 (a). That means that for adenosine concentration of 1 mM the surface was not completely covered by the adenosine molecules for potentials negative to the peak pairs Ad$_2$/Ad'$_2$. Positive to these peaks no change could be obtained, which means that at positive potential region the coverage of the surface remains the same as for the lower molecular concentration. Worth noting is that even for a higher concentration no needle peak regarding to formation/dissolution of the physisorbed condensed film was observed. Again, a decrease of

the temperature in a range between 20 and 5 °C implied a decrease in the current of the cathodic peak Ad'$_1$, while for positive potentials any change was observed.

To explain the absence of a chemisorbed film at negative potentials as was obtained for adenine nitrogen base, we assume that the adenine group must be orientated, in relation to the electrode surface, in a different position as was in the adenine case. The presence of the sugar group attached to the N(9) position of adenine may change the adsorbed orientation of adenosine. SERS experiments performed for NAD (nicotinamide adenine dinucleotide) on gold electrode has suggested that, at negative potentials, the adenine moiety present in the NAD is flat orientated and binds the electrode surface with its π-electrons [118]. The obtained spectra at negative potentials showed that the both extremities of NAD molecule, i.e., the adenine base and the nicotinamide, must be adsorbed at the electrode surface. This characteristic is incompatible with adenosine adsorption; otherwise the charge-transfer complex should be obtained as was for the adenine base. The NAD can hardly be compared with adenosine. For this reason at present we do not know if the sugar group is pointing toward to the solution side or if it is adsorbed on the electrode surface.

The adenosine orientation could explain the absence of charge-transfer complex of the π*-orbital of adenine and the electrode surface at negative potential. As the orientation of adenine is changed due to the presence of the sugar group, it cannot bind the surface with its π*-orbital. However, the shape of the signal pair Ad$_2$/Ad'$_2$ reminds strongly a redox coupling where both components are irreversibly adsorbed, i.e., chemisorbed. More experiments are necessary to conform this proposal.

6.3 COADSORPTION BETWEEN ADENOSINE AND THYMINE ON AU(111)

Considering the interaction between the adenine and thymine complementary bases on Au(111) discussed in Chapter 4, in the following we investigate the effect of the sugar moiety on the interaction between the bases. Figure 6.4 shows the voltametric profile for a fixed thymine concentration (20 mM) at different adenosine concentrations.
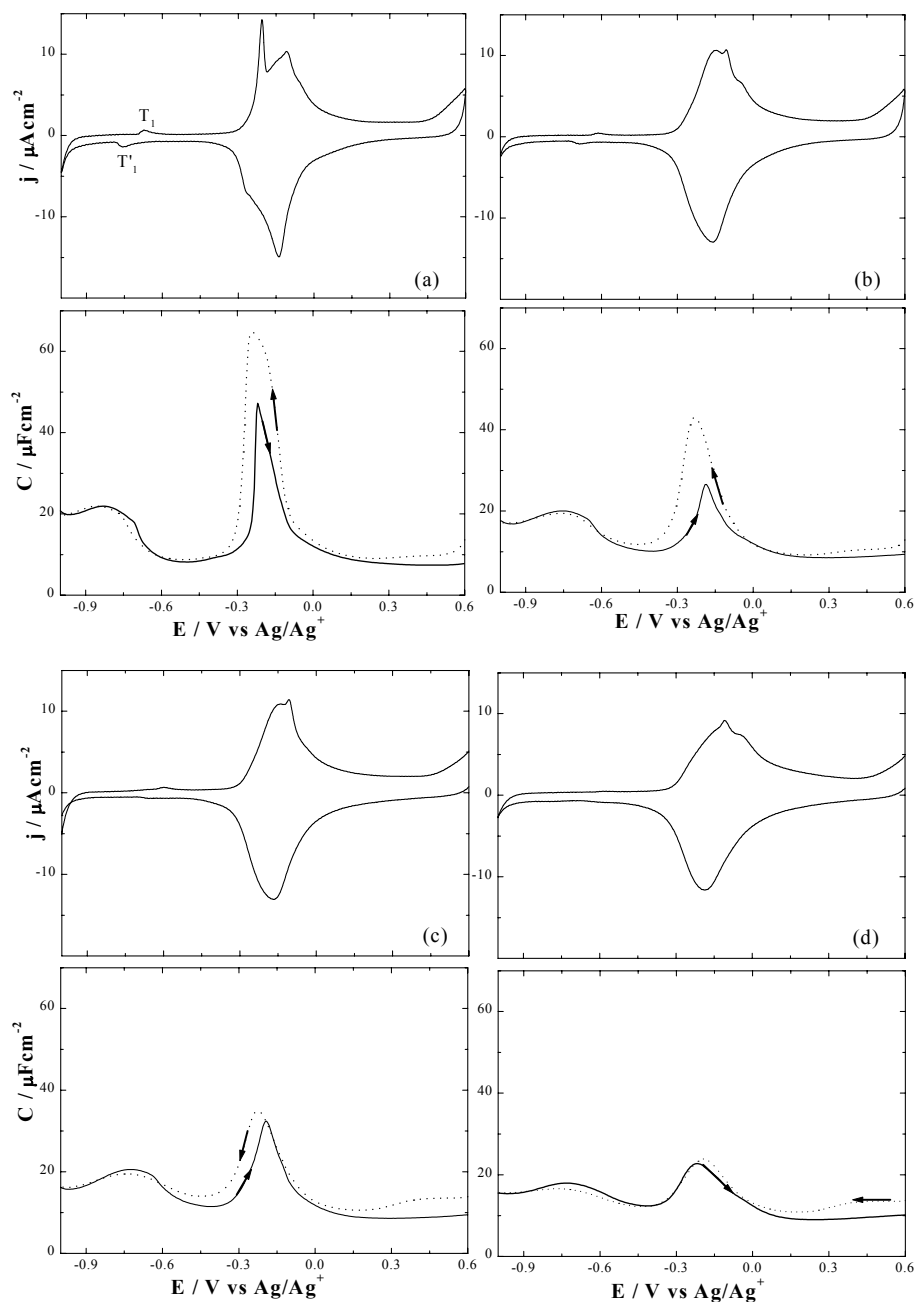
Figure 6.4- Cyclic voltammograms of thymine 20 mM + adenosine 0.05 mM (a), thymine 20 mM + adenosine 0.5 mM (b), thymine 20 mM + adenosine 1 mM (c), thymine 20 mM + adenosine 2 mM (d) and the respective capacity curves. Supporting electrolyte: 0.1 M NaClO$_4$ (pH = 2). Sweep rate: v = 50 mV/s (cyclic voltammograms) and v = 5 mV/s (capacity curves). Perturbation frequency and amplitude when measuring the capacity curve: 20 Hz, and 3 mV, respectively. T = 20 °C.

Figure 6.4 (a-d) shows the influence of the adenosine concentration on the adsorption behavior of thymine. For every concentration ratio of thymine / adenosine the CVs as well the capacity-potential curve is shown. Figure 6.4 (a) the CV of a solution that is 400 times higher in thymine than in adenosine. One recognizes the shape of the cyclic voltammogram being very similar to that observed for solution containing only thymine. The small needle peaks indicate the formation of the physisorbed thymine layer, which undergoes at more positive potential the phase-transition to the chemisorbed thymine film. Increasing the concentration of adenosine, the characteristics of the CV becomes more and more similar to adenosine, i.e., adenosine is adsorbed. But at the anodic side of the broad signal (Figure 6.4 (d)) there is a shoulder, which may be due to chemisorbed thymine. This observation strongly indicates that both molecules are adsorbed on the electrode surface, however it seems that there is no interaction between them in the adsorbed state.

6.4 THYMIDINE

The structure of pyrimidine nucleosides is very similar to purine nucleosides. The pyrimidine is linked to the sugar group by a β-N-glycosyl bond to D-ribose (nucleoside) or 2-deoxy-D-ribose (deoxyribonucleoside). The N(1) nitrogen of the pyrimidine is attached to the sugar C-1' carbon atom [119].

6.4.1 ADSORPTION OF THYMIDINE ON AU(111)

Figure 6.5 shows the adsorption behavior of thymidine 20 mM on Au(111) and the respective capacity curves for temperature between 20 and 5 °C. In comparison to adenosine, the maximum of the signals is not shifted on the potential scale. However, the shape has changed in comparison to the adenosine peaks. The signals of the thymidine are narrower and show a structural feature around –0.2 V, which reminds a needle peak.
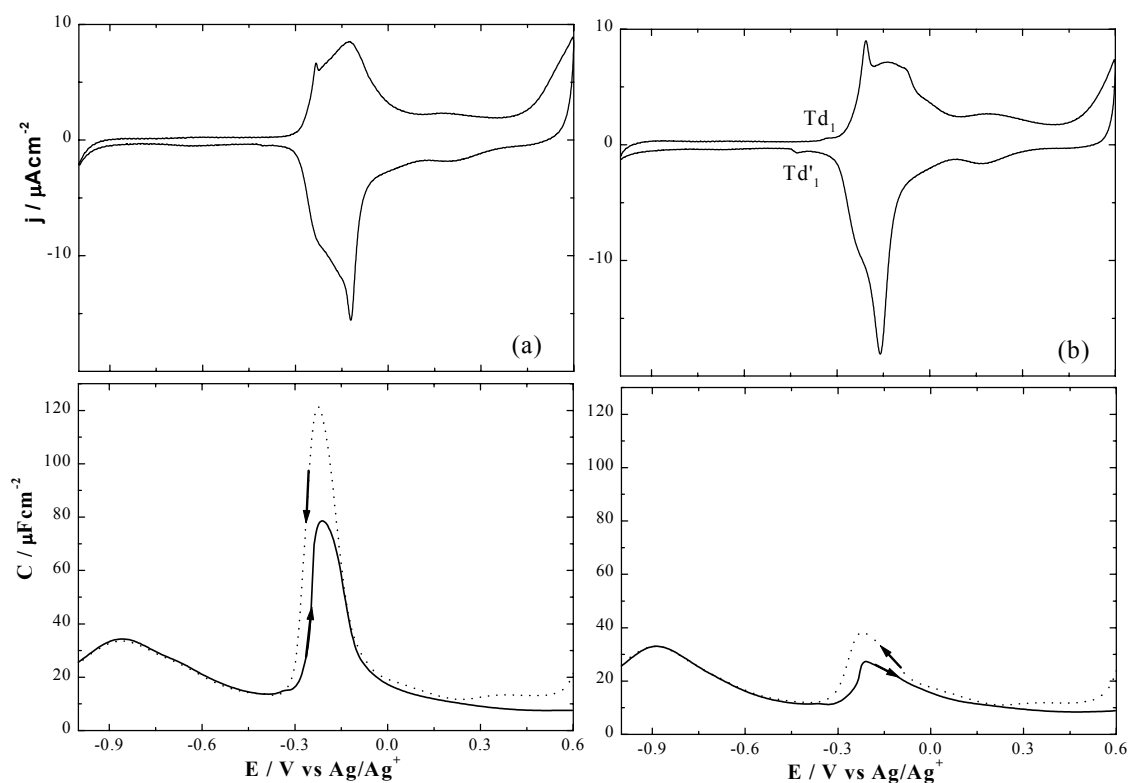
Figure 6.5: Cyclic voltammograms of thymidine 20 mM at T = 20 °C (a), T = 5 °C (b) and the respective capacity curves. Supporting electrolyte: 0.1 M NaClO$_4$ (pH = 2). Sweep rate: v = 50 mV/s (CVs) and v = 5 mV/s (capacity curves). Perturbation frequency and amplitude when measuring the capacity curves: 20 Hz, and 3 mV, respectively.

As can be seen further, the presence of a sugar group changed the CV profile compared to the thymine base. For thymidine concentration of 20 mM no well resolved phase-transition peak was obtained, but in the cathodic scan the presence of a small peak can be seen at -0.40 V (Td'$_1$) for T = 20 °C. A decrease of the temperature to 5 °C caused a potential change in this small peak to -0.42 V (Td'$_1$), at the same time that a small positive peak is formed at -0.33 V (Td$_1$) showing the formation of thymine physisiorbed film in a short potential range. This indicates that the stability of the condensed film is high at lower temperature as for thymine base.

Since we could not see any well solved peaks for the concentration of thymidine 20 mM, the concentration was increased to 200 mM (Figure 6.6) to try to obtain more defined peaks.
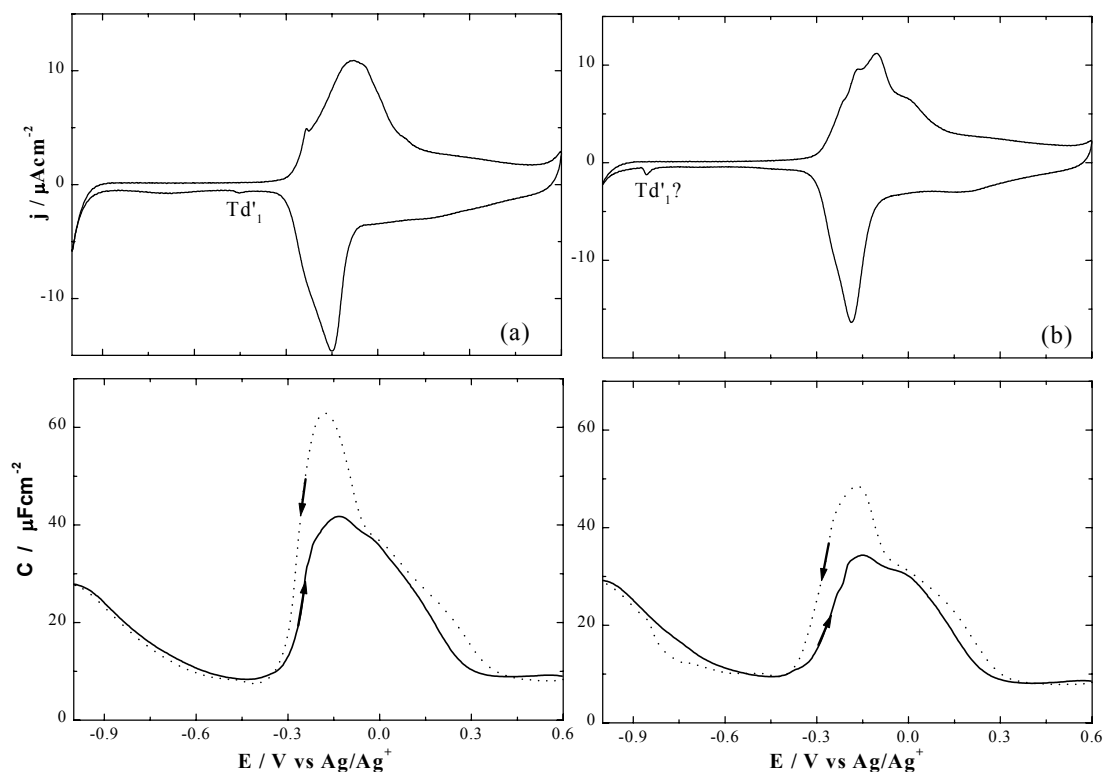
Figure 6.6: Cyclic voltammograms of thymidine 200 mM at T = 20 °C (a), T = 5 °C (b) and the respective capacity curves. Supporting electrolyte: 0.1 M NaClO$_4$ (pH = 2). Sweep rate: v = 50 mV/s (CVs) and v = 5 mV/s (capacity curves). Perturbation frequency and amplitude when measuring the capacity curves: 20 Hz, and 3 mV, respectively.

Quite surprisingly, at high thymidine concentration the small negative phase-transition peak did not show an increasing in the current profile, but comparing the peak potential with the concentration of 20 mM it changed its position to about 0.4 V more in a negative potential. Interesting is to note the CV profile at negative potential when the temperature is decreased to 5 °C. At a first glance it seems that the negative peak (Td'$_1$) changed its potential position more than 0.4 V negative as for T = 20 °C. Of course, this phenomenon cannot be due to a simple temperature decrease, since the range of the potential variation is too pronounced. In order to provide a further insight into such an abnormal behavior, the system was studied at slower sweep rate. The corresponding CVs are depicted in Figure 6.7.
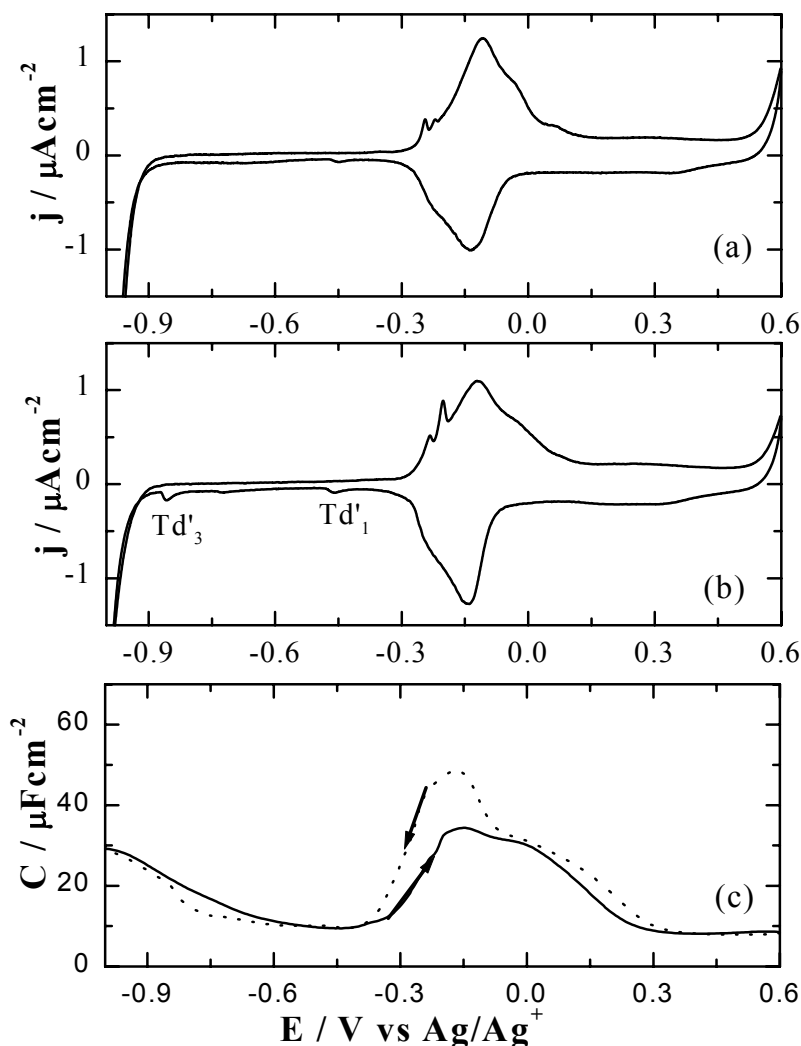
Figure 6.7: Cyclic voltammograms of thymidine 200 mM at T = 20 °C (a), at T = 5 °C (b), and capacity curves at T = 5 °C (c). Supporting electrolyte: 0.1 M NaClO$_4$ (pH = 2). Sweep rate: v = 5 mV/s (in any case). Perturbation frequency and amplitude when measuring the capacity curves: 20 Hz, and 3 mV, respectively.

For thymidine 200 mM at T = 20 °C with lower scan rate any change was obtained. On the other hand, at T = 5 °C one can clearly see the presence of two negative peaks. It becomes clear now that the peak at -0.85 V in Figure 6.6 (b) is a new peak. The absence of anodic peaks regarding to formation of the physisorbed film can be due to the lower rate of the film formation in comparison with the film dissolution (Td'$_1$). The formation of the new peak Td'$_3$ may be due to a reorientation of the thymidine molecules, which is indicated in the capacity curve in the back scan (Figure 6.7 (c)) by the hump at the same potential (-0.85 V) as for the peak Td'$_3$ in the voltammogram (Figure 6.7 (b)).

Comparing the behavior of thymidine adsorption with the nitrogen base thymine, the presence of the sugar group influences mainly the adsorption on the negative potential region, once no appreciable difference was observed at positive potentials. At concentration of 20 mM and 200 mM thymidine did not show any well-defined phase-transition peak.

We assume that the reason for the absence of the well-solved phase–transition peaks even in a considerably high thymidine concentration of 200 mM is that the critical concentration required for the phase-transition and consequently to form the physisorbed condensed film at very negative potentials is not reached. To form a new phase, the molecules must first aggregate and form cluster and then expand into a new phase [76]. The sugar moiety also may causes a steric hindrance between the adsorbed molecules and the critical concentration is reached at more positive potentials. As a consequence, the condensed film is formed in a very small potential range when compared to thymine base.

6.5 COADSORPTION BETWEEN THYMIDINE AND ADENOSINE ON AU(111)

The CV for adenosine and thymidine in the same solution are shown in Figure 6.8. The adenosine-thymidine solution shows that the small cathodic peak obtained for thymidine could not be seen. The region related to formation/dissolution of the chemisorbed film is observed at the same potentials with respect to thymidine and remains almost unchanged. Increasing the adenosine concentration to 2 mM and decreasing a little the thymidine concentration, almost any change on the shape was obtained. That means that the electrode surface is covered by thymidine molecules but no interaction between adenosine and thymidine could be found. Two may be the reason for this behavior: (a) the high thymidine molecules concentration in the solution or (b) due to the orientation of both molecules in the negative potentials that do not favor the formation of hydrogen bonding between them. The showed results indicate that no interaction between adenosine and thymidine could be found, at least in the parameter range investigated here.
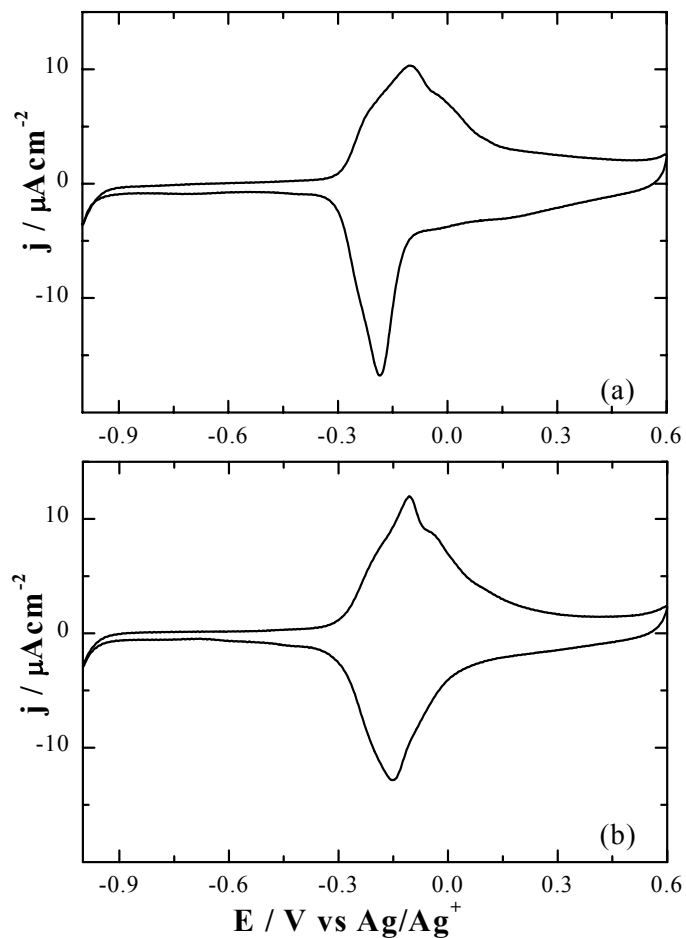
Figure 6.8: Cyclic voltammograms of thymidine 190 mM + adenosine 0.005 mM (a), thymidine 171 mM + adenosine 2 mM (b). Supporting electrolyte: 0.1 M $NaClO_4$ (pH = 2). Sweep rate: v = 50 mV/s. T = 20 °C.

6.6 SUMMARY

The nucleosides adenosine and thymidine showed a different adsorption behavior on Au(111) when compared with the adsorption behavior of the bases adenine and thymine on the same electrode surface. Both nucleosides are strongly adsorbed at the electrode; however, there are no results, which support the extreme of an ordered condensed film of these molecules. The reason for it may be the different orientation or adsorption state of the nucleosides on the charged surface.

For the coadsorption between adenosine and thymidine no interaction was found. The reason may be due to the presence of the sugar group, which changes the adsorption state of the molecules on the electrode surface.