CHAPTER 5: BROMOURACIL, ADENINE AND GUANINE

In this present chapter we show the adsorption behavior of bromouracil non-standard base and guanine standard base. The adsorption of bromouracil is similar to thymine (chapter 4), but guanine presents an interesting adsorption behavior depending on the solution pH value. The coadsorption behavior of bromouracil with adenine and guanine on Au(111) surface is also presented.

5.1 BROMOURACIL

5.1.1 GENERAL ASPECTS

It has been shown [101] that DNA double strand are stabilized by the so-called complementary base pairs A-T and C-G, which duplicates to transmit the genetic information. If one base in a complementary base pair is replaced by another base or by a mimic, mutation can occur. In this way, mutation can be defined as a heritable effect in the genetic material (DNA), which can be caused by different ways as base pairs substitution or base alterations. Base alteration is a spontaneous mutation, and occurs as a result of natural process in the cell. These structural alterations are called *tautomerism*, i.e., the bases, which exist in different tautomeric forms can interconvert. In the case of base pair substitutions, there are two possibilities: (1) *transition,* where purine is replaced by purine and the pyrimidine by pyrimidine and (2) *transversion*, where the purine is replaced by a pyrimidine and a pyrimidine is replaced by a purine. The consequences of these base replacement in the gene generally depends on the substitution and its location. In case that the substitution takes place by an external agent, the mutation is generally called *mutagen*. This is the case for example if bromouracil (has a Br at position 5 instead of methyl group in thymine), that resembles thymine and is incorporated into the DNA and forms a non-complementary pair with adenine as illustrated in Figure 5.1. The resulting unusual base pairs are called non-standard base pairs.
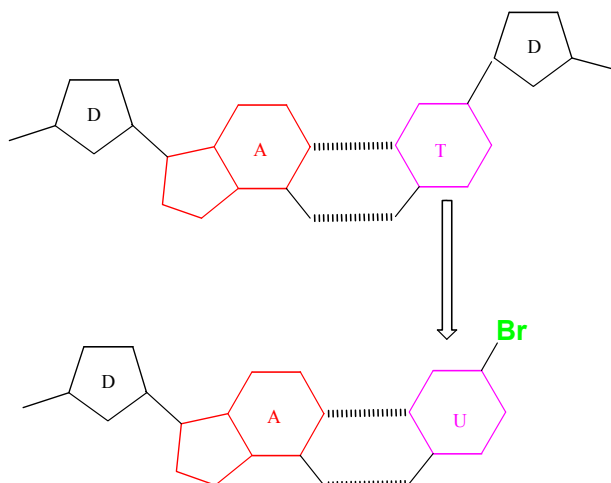
Figure 5.1: Replacement from thymine to bromouracil in the genetic code.

It is known from *ab initio* calculations that bromouracil forms a more stable hydrogen bonds with guanine than with adenine [102]. During the DNA duplication a mispairing occurs, which can cause cancer disease. On the other hand, the substitution of thymine by 5-halouracil in the genetic sequence of cellular DNA leads to greater sensitivity to ionizing radiation [103,104] without changing the normal gene expression in the unradiated cells. The ionizing radiation has an important role in cancer therapy, which produces a range of damage in the tumor cells due to the production of free radicals of water. Free radicals have unpaired electrons with higher reactivity that interact with the DNA. Thus, the X-ray application causes DNA damage, which may result in the block of the cell division or its death.

5.1.2 TAUTOMERISM AND pK VALUE

Heterocyclic molecules generally yield a mixed population of tautomeric forms in solution in rapid equilibrium when hydrogen atoms are able to migrate within the molecule. The tautomeric forms for bromouracil are shown in Figure 5.2.
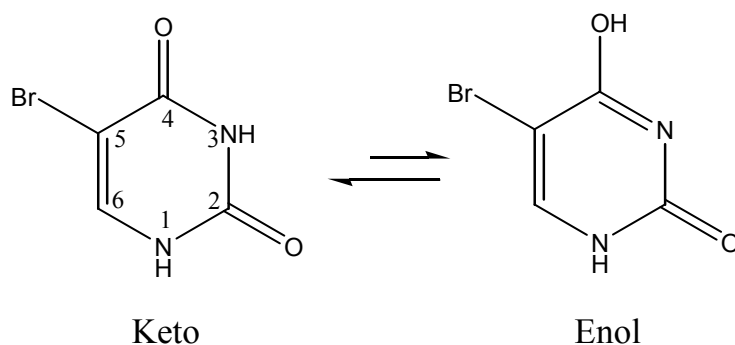
Figure 5.2: Tautomeric forms and ionization constant of bromouracil.

At alkaline pHs, the hydrogen in the N(3) for bromouracil is removed, indicating that the amide-like hydrogen is rather acidic, whereas the ring nitrogen at N(1) is basic, as depicted Figure 5.3.
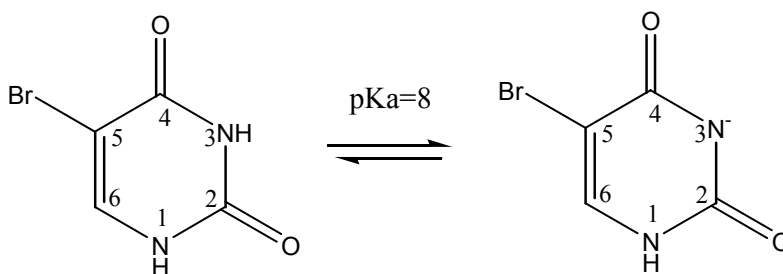

Figure 5.3- Ionization constant of bromouracil.

It has been suggested by Watson and Crick in a classical paper [101] that spontaneous mutation might be due to the occurrence of a purine-pyrimidine pair with a base in one of its minor tautomeric form.

5.1.3 ADSORPTION OF BROMOURACIL ON AU(111)

The adsorption behavior of bromouracil on the Au(111) electrode is comparable with the pyrimidine thymine. When varying the applied potential and the adsorbate concentration, one can recognize four different adsorption states (Figure 5.4). As the qualitative properties for bromouracil are very similar to thymine molecules, we just summaries the regions briefly: in the region (I) the molecules are random adsorbed at low coverage; in region (II) a two

dimensional condensed film is formed by a first order phase-transition, as indicated by the sharp needle peaks; in region (III) a complicated reorientation and a partial charge transfer is assumed; in region (IV) the molecules are associated in a highly ordered chemisorbed phase. The only difference between thymine and bromouracil that should be pointed out is related to the dissolution kinetic of the chemisorbed bromouracil phase. Scanning from positive to negative potentials, the dissolution of the chemisorbed and the formation of the physisorbed state occurs at different potentials, whereby the transformation is characterized by at least three different stages. At this point it is interesting to recall that the transformation between state IV and II for thymine adsorbed layers occurs also in three stages. But in contrast to bromouracil, it takes place parallel, or, the potentials are close located that only in the current transients the process can be distinguished. In the capacity curve, only two adsorbate states can be identified, whereby between the forward and the backward scans no hysteresis is observed.
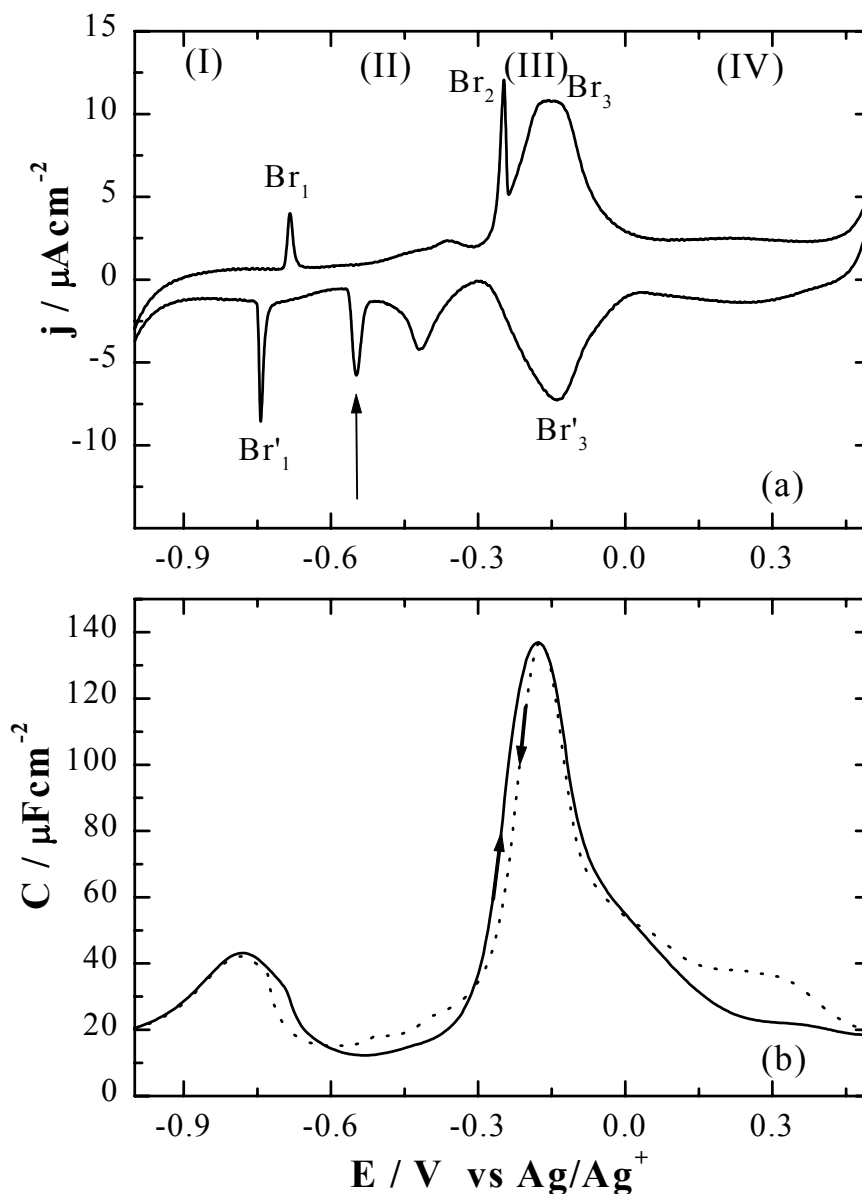
Figure 5.4: Cyclic voltammogram of 5mM bromouracil (a) and capacity curves (b). Supporting electrolyte: 0.1 M $NaClO_4$ (pH = 2). Sweep rate: v = 50 mV/s (a) and v = 5 mV/s (b). Perturbation frequency and amplitude when measuring the capacity curve: 80 Hz, and 3 mV, respectively. T = 20 °C.

Figure 5.5 depicts the voltammetric response of Au(111) electrode in the presence of 5 mM bromouracil at different pH values. It becomes clearly evident in this figure that the adsorption behavior of bromouracil has a strongly dependence on the pH values. In the pH range between 2 and 8, the stability region of the condensed physisorbed layer becomes smaller, as is seen by the significant shift of the needle peaks in the positive direction. The

stability of the chemisorbed becomes broader, and the peaks corresponding to region III shift towards negative potentials.
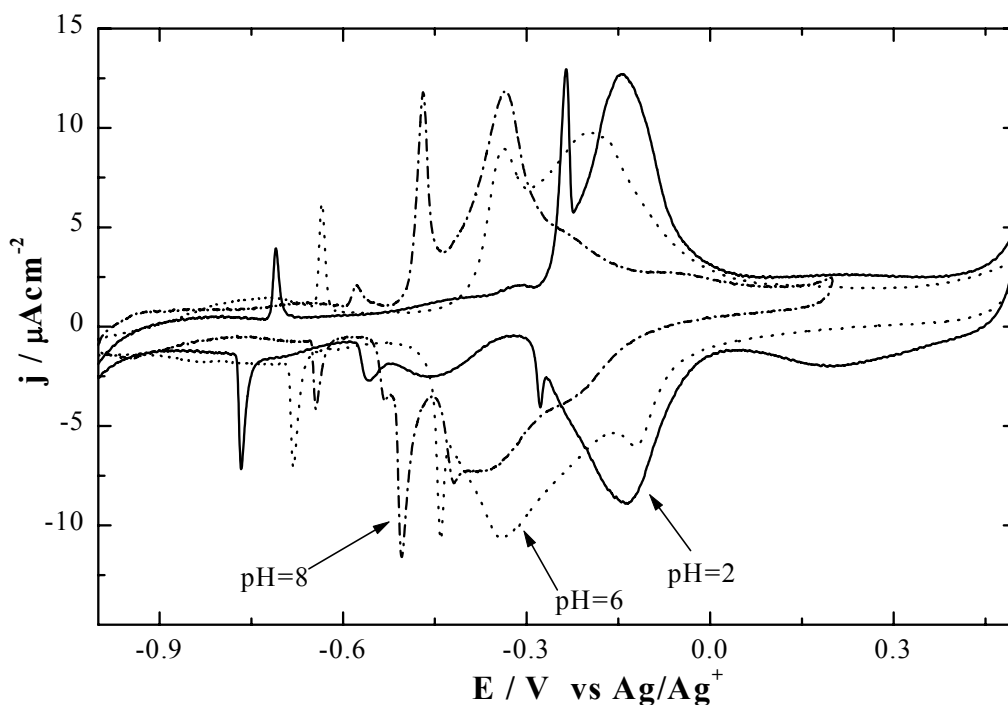

Figure 5.5- Cyclic voltammogram of 5 mM bromouracil at different pH values. Supporting electrolyte: 0.1 M NaClO$_4$. Sweep rate: v = 50 mV/s. T = 20 $^{\circ}$C.

Obviously, the same explanation as for the pH dependence of thymine is given for bromouracil, which assume a deprotonation of the molecule accompanied by a partial charge-transfer from the molecule to the electrode, if it is transformed from the physisorbed into the chemisorbed state. Cunha *et al.* [105] proposed the structure of adsorbed bromouracil at negative potentials on Au(111) in acidic solution by STM inference. In this model, bromouracil form rows of dimmers and tetramers. Moreover, the model proposed that the tetramers are probably stabilized by hydrogen bonding within the tetramers and between the adjacent tetramers, making the structure very stable (Figure 5.6). In the same way, the dimmers are stabilized by hydrogen bondings within the dimmers. Outside these rows of dimmers and tetrameters no free sites for hydrogen bonds are available.
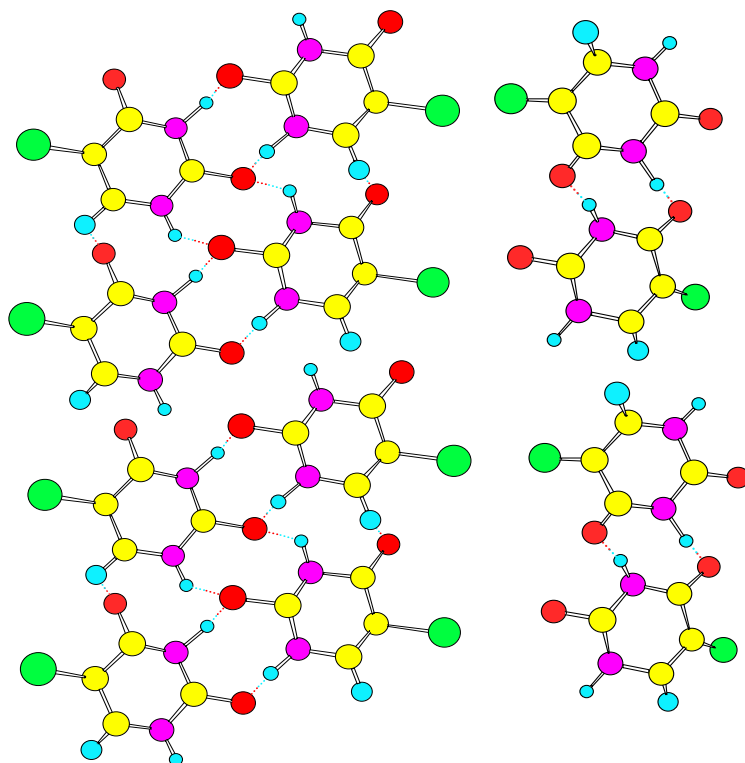
Figure 5.6: Model of bromouracil structure at negative potentials (after ref. [105]).

In Figure 5.7, the temperature effect of the adsorption of bromouracil at pH = 8 is shown. The needle peaks Br_1/Br'_1 indicates the phase-transition in the physisorbed phase disappeared at 20 °C. At 5 °C, the stability region of the condensed physisorbed phase becomes broader as for 10 °C, as inferred by the shift of the needle peaks towards negative values, in accordance with the temperature behavior of physisorbed molecules [52]. At more positive potentials, the position of the peak pairs Br_2/Br'_2 and Br_3/Br'_3 are nearly temperature independent. It seems that the transformation from state II to IV becomes faster with decreasing the temperature, but in a more "compact" form at more negative potentials, as obtained for thymine (see Figure 4.4 in Chapter 4).
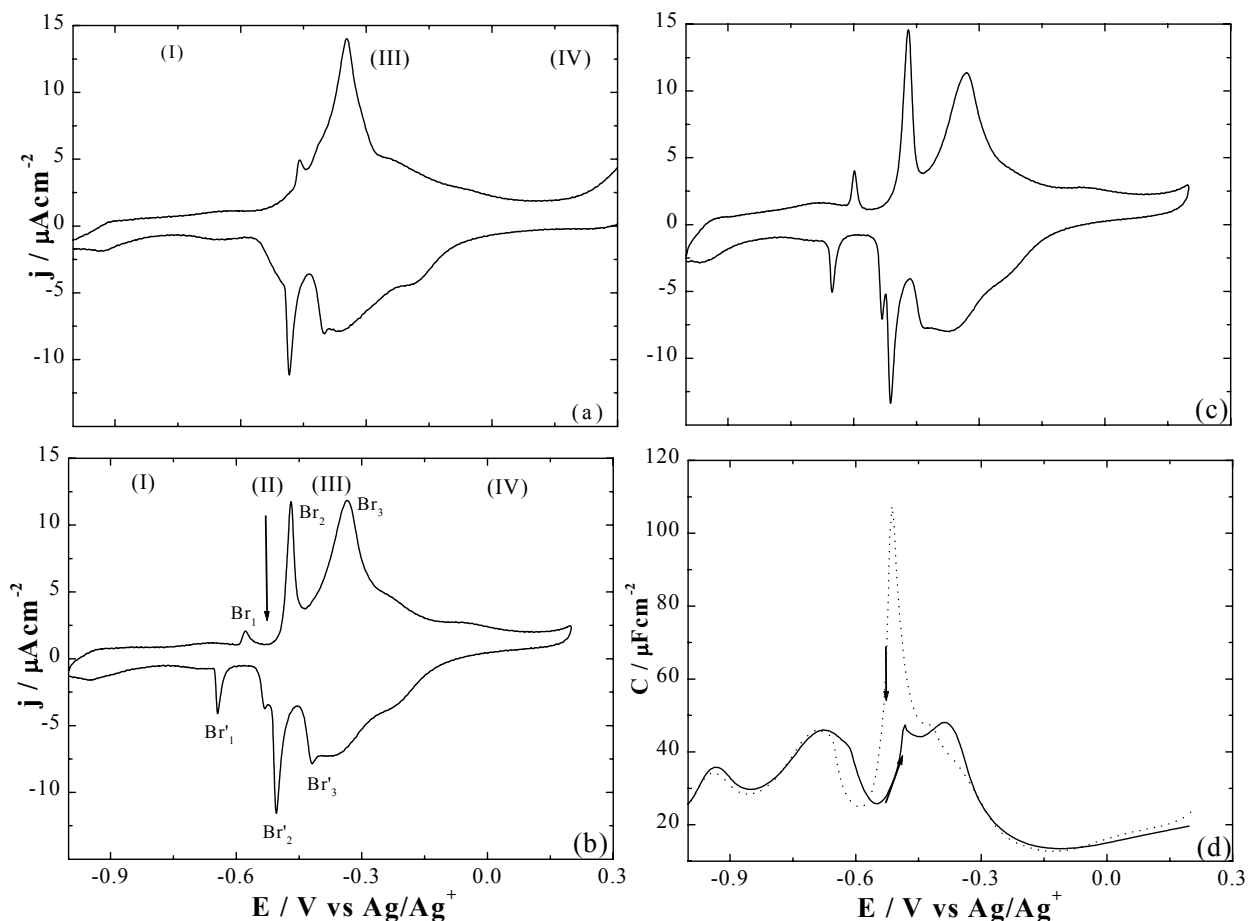
Figure 5.7- Cyclic voltammogram of 5 mM bromouracil at T = 20 °C (a), T =10 °C (b), and T = 5 °C (c). Supporting electrolyte: 0.1 M NaClO$_4$ (pH = 8). Sweep rate: v = 50 mV/s. Perturbation frequency and amplitude when measuring the capacity curve: 100 Hz, and 3 mV, respectively, v = 5.0mV/s.

5.2 GUANINE

5.2.1 GENERAL ASPECTS

Guanine is a purine as adenine and is present in both DNA and RNA molecules. In the double strand or in the cell processes as the DNA replication and gene expression, guanine forms Watson and Crick base pairs with cytosine. The base pairing is formed via the amino group, the N(1)H and the carbonyl group of guanine by hydrogen bonding with the carbonyl group, the N(3) and the amino group of cytosine, respectively.

Guanine is the second purine most commonly found in the nucleic acids. The guanine nucleotides, like adenine nucleotides, are involved in intermediary metabolism and are also found in many animals' excrements and tissues [106]. There are certain synthetic purines of considerable interest in cancer chemotherapy, as 6-thiopurine, one of the most effective drugs available for the treatment of a number of types of leukemia.

5.2.2 TAUTOMERISM AND pK VALUE

As already seen in Chapter 4, for thymine and adenine DNA bases, guanine like all the commonly bases is capable to exist in tautomeric keto and enol equilibrium, as shown in Figure 5.8. The keto form is so strong favored, that is difficult to detect even some traces of the enol form at the equilibrium.
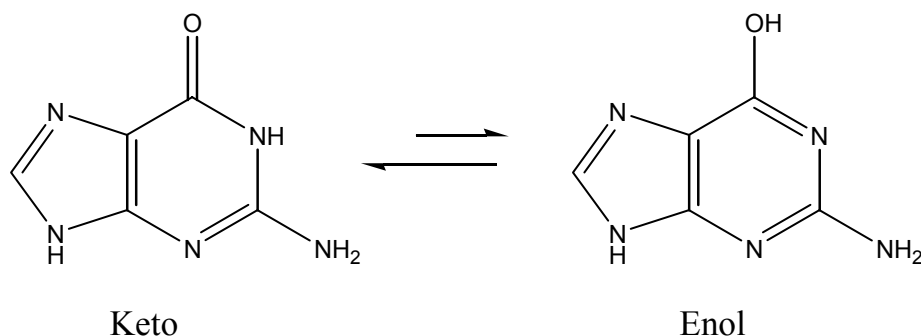

Keto   Enol

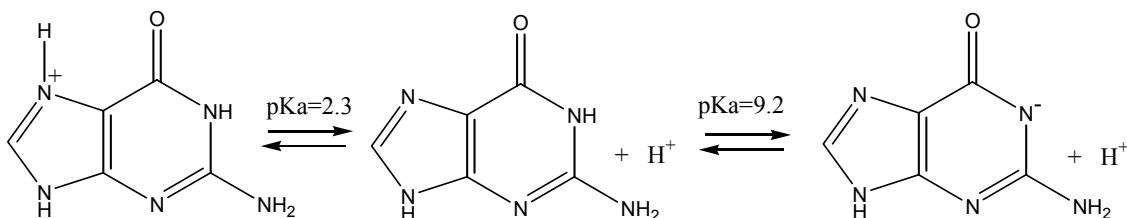**Figure 5.8:** Tautomeric forms for guanine.

Figure 5.9: Ionization constant for guanine.

5.2.3 ADSORPTION OF GUANINE ON AU(111)

Guanine is one of the two principal purine bases found in the nucleic acids and is believed to play an important role in oxidation of DNA by various types of oxidants and free radicals. Guanine adsorption has been studied at glassy carbon electrode concluding that its oxidation occurs in two steps including formation of dimmers [107,108]. On polycrystalline gold electrodes, the kinetic data suggested that the electrochemical oxidation of guanine depended strongly on the applied potential sweep rate [109].

It is know from the theoretical approach [110], that guanine is the most challenging nitrogen base due to the different characteristics regarding to the numerous tautomeric forms, protonation and deprotonation of the molecule. In the following it is discussed the effect of increasing the solution pH.

The guanine adsorption on Au(111) gives a surprising and very interesting effect depending on the pH of the solution. Guanine is a purine as adenine, but its adsorption kinetics is completely different when compared with adenine at the same pH value. It should be mentioned that at pH = 2 adenine forms a charge-transfer complex involving the π^*-orbitals of adenine and the d-orbitals of gold electrode at negative potential and the physisorbed film is formed at positive potentials (see Chapter 4, Figure 4.24). In contrast, the adsorption of guanine is similar to a pyrimidine, as can be seen in Figure 5.10.
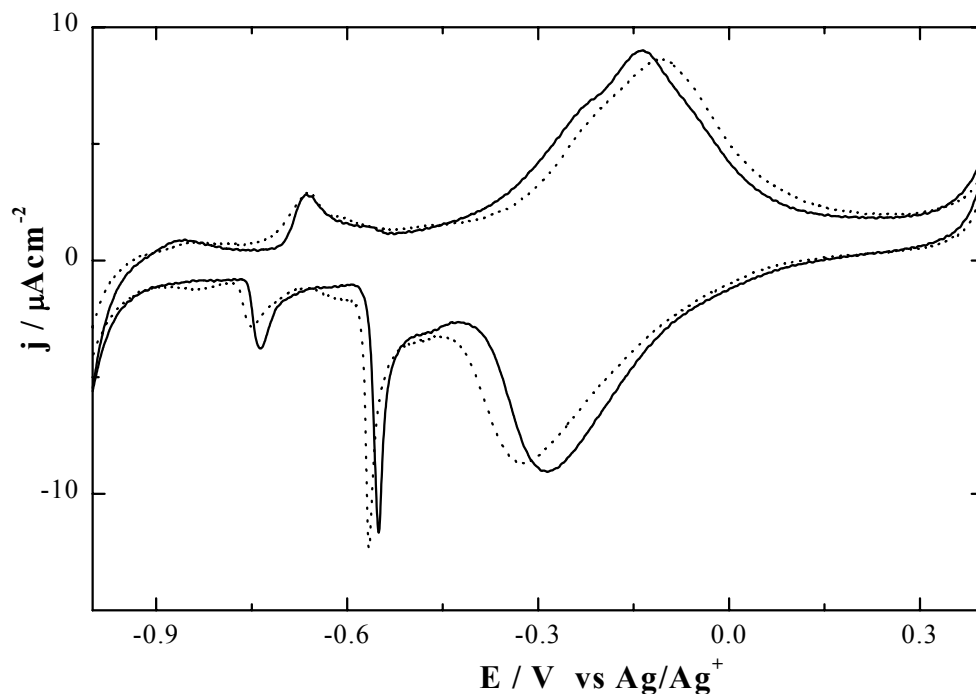
Figure 5.10- Cyclic voltammogram of 0.1 mM guanine at T = 20 °C (solid line) and 10 °C (dotted line). Supporting electrolyte: 0.1 M NaClO$_4$ (pH = 2). Sweep rate: v = 50 mV/s.

The sharp peaks at negative potentials indicate that a phase transition takes place, which depends on the temperature, as the formal scenario found for pyrimidines. Thus, it seems to be plausible to assume that the interaction between guanine and the gold surface is less stable than the adsorption of adenine at the gold surface. Guanine is mobile enough in this potential range and is able to form a monolayer via two dimensional nucleation and growth process. The peak pairs at about –0.3 V to –0.1 V is similar to the peak pairs indicating the transition from II to IV shown in Figure 4.3 (Chapter 4) for the adsorption of thymine.

Increasing the pH = 6 (Figure 5.11), the shape of the CV becomes very different when compared with the CV at pH = 2 shown in Figure 5.10. No phase-transition peaks could be seen at negative and positive potentials. Instead of the needle peaks obtained at pH = 2, the CV shows more broadened peaks at negative potentials. In the capacity curves three minima can be observed, whereby the capacity is lowered between –0.6 and –0.3 V.
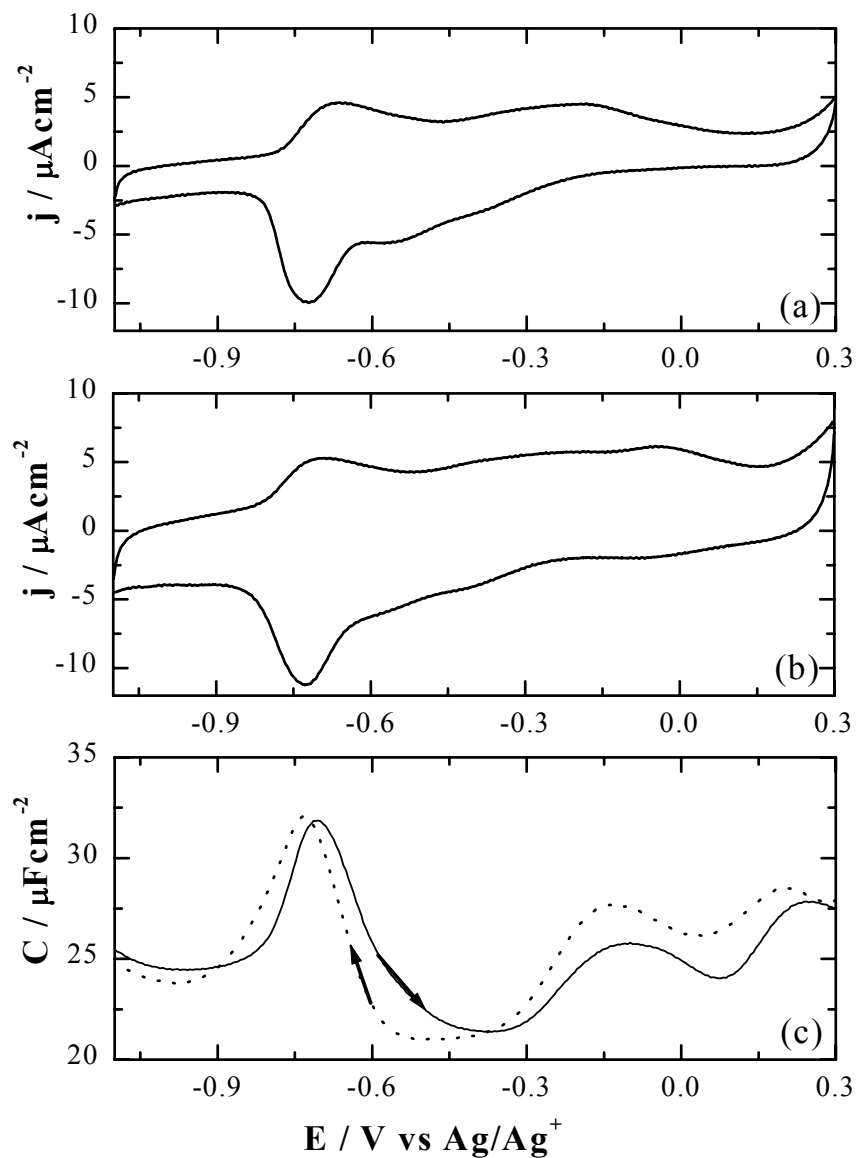
Figure 5.11- Cyclic voltammograms of guanine 0.02 mM (a), 0.05 mM (b), and capacity curves for guanine 0.05 mM (c). Supporting electrolyte: 0.1 M NaClO$_4$ (pH = 6). Sweep rate: v = 50 mV/s ((a) and (b) and v = 5 mV/s (c). Perturbation frequency and amplitude when measuring the capacity curve: 100 Hz, and 3 mV, respectively. T = 10 ^{o}C.

Experiments with guanine concentration of 0.05 mM at T = 20 °C and 10 °C are depicted in Figure 5.12. An increase of the guanine concentration does not reveal a significant change regarding the peaks, indicating that the electrode surface is completely covered by the guanine molecules. Also lowering the temperature the peak position did not change, showing a strong interaction between guanine and the gold surface.
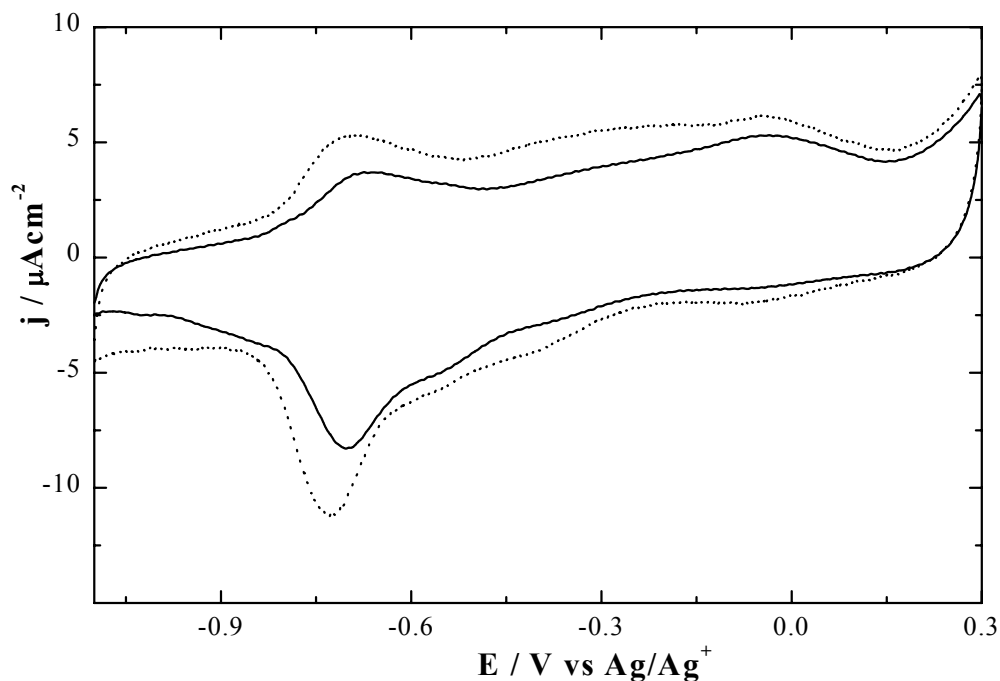
Figure 5.12- Cyclic voltammogram of guanine 0.05 mM at T = 20 °C (solid line) and T = 10 °C (dotted line). Supporting electrolyte: 0.1 M NaClO$_4$ (pH = 6). Sweep rate: v = 50 mV/s.

The effect of higher pH at different guanine concentration is given in Figure 5.13. The experiments were performed at pH = 8. In this case, guanine adsorption on Au(111) presents better resolved and more defined peaks at negative potentials when compared with guanine at pH = 6. The two peaks pairs G$_1$/G'$_1$ and G$_2$/G'$_2$ at higher current, whereby the peak position shifts only slightly comparing with pH = 6.
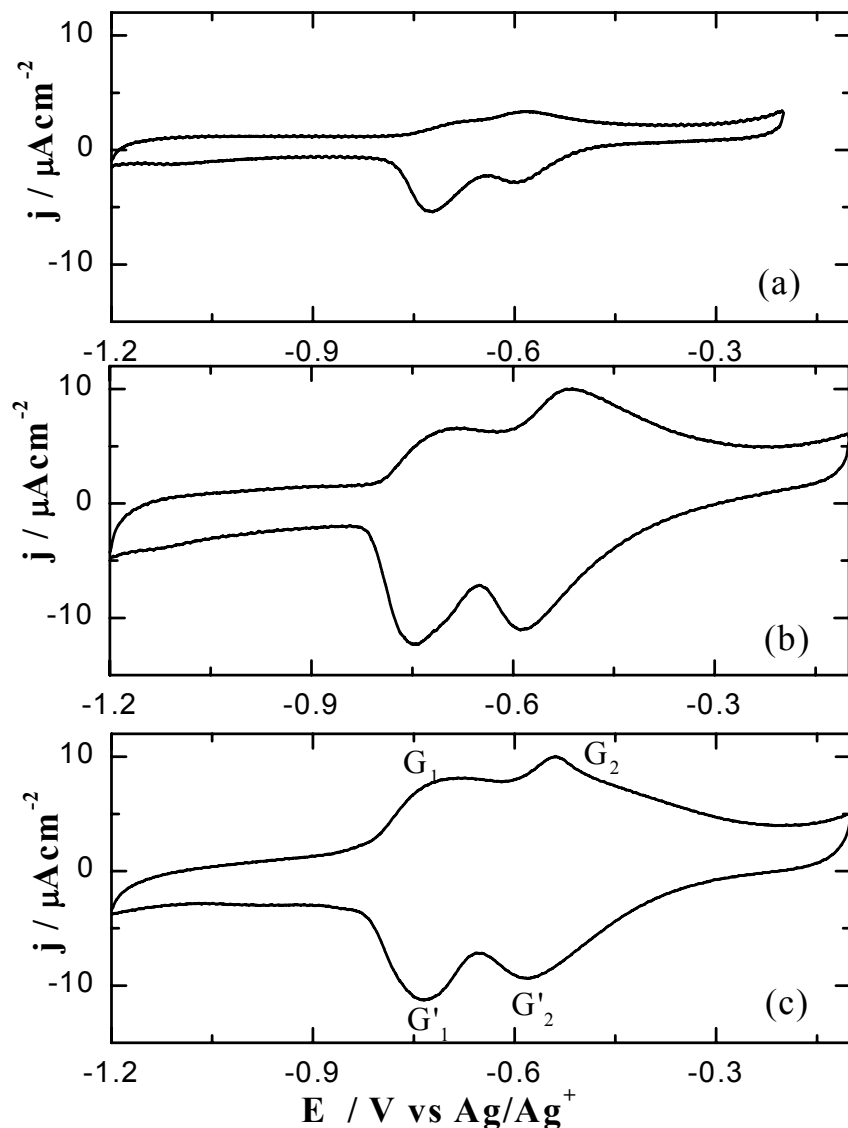
Figure 5.13- Cyclic voltammograms of guanine at 0.005 mM (a), 0.03 mM (b) and 0.05 mM (c). Supporting electrolyte: 0.1 M NaClO$_4$ (pH = 8). Sweep rate: v = 50 mV/s. T = 20 ^{o}C.

The influence of the temperature on the adsorption was studied and the results are depicted in Figure 5.14. The positions of the peak pairs depends slightly on the bulk concentration of guanine in a range between 0.05 and 0.005 mM, only an increasing in the current density accompanying the increasing of the guanine concentration for temperature in a range between 20 °C and 5 °C. We assume that the peaks correspond to a formation of the chemisorbed film, or, at least, a strong adsorbed monolayer of guanine. Once these peaks are more negative located as the chemisorption peak found for pH = 2, we assume that the

interaction between guanine and the gold surface at negative potentials is similar as found for adenine-gold interactions at pH = 2. Contrary to our expectations, the same behavior as for adenine at pH = 2 was found for guanine at pH = 8 (Figure 5.14).
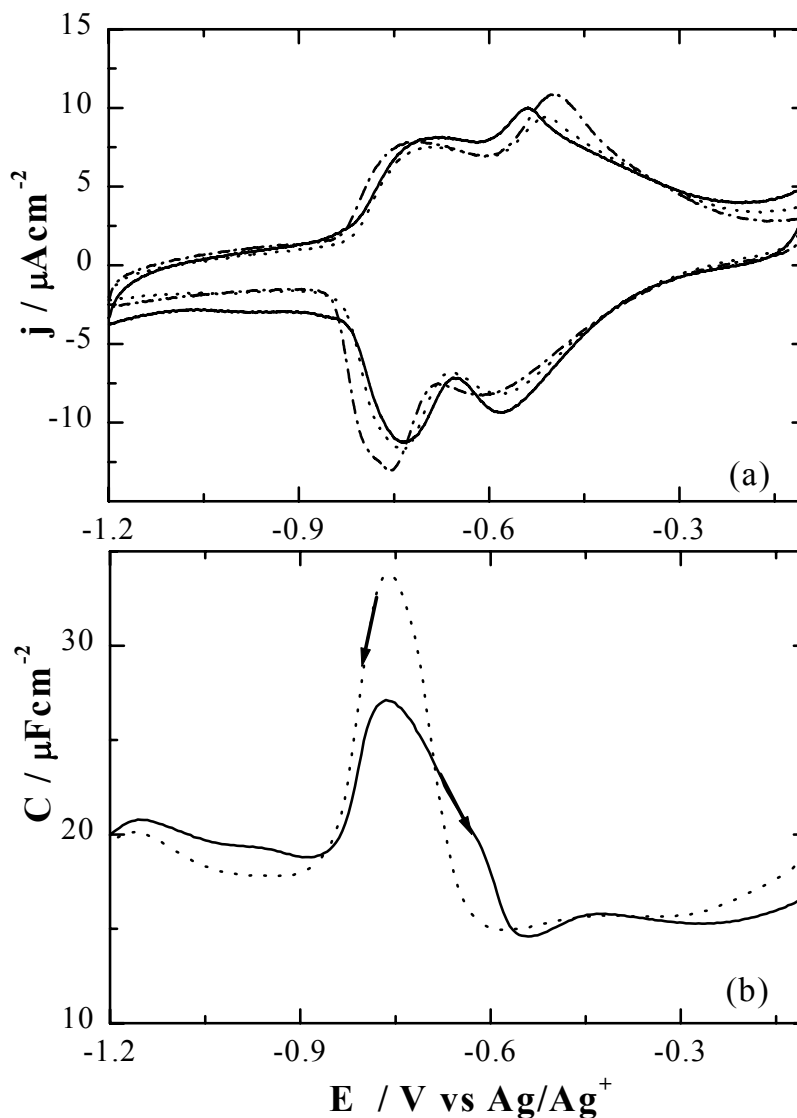

Figure 5.14- Cyclic voltammograms for guanine at 0.05 mM at T = 20 °C (solid line), T = 10 °C (dotted line) and T = 5 °C (dotted dashed line) (a). Capacity curve at T = 20°C (b) and identical remaining conditions than in (a). Supporting electrolyte: 0.1 M NaClO$_4$ (pH = 8). Sweep rate: v = 50 mV/s (a) and v = 5 mV/s (b). Perturbation frequency and amplitude when measuring the capacity curve: 100 Hz, and 3 mV, respectively.

One possible explanation for this, say, counter intuitive behavior can be given by considering the pK values of the guanine molecule, since the nitrogen bases undergo

protonation in acid and deprotonation in alkaline solution. At lower pH values, guanine is protonated at N(7), at the five membered ring, rather than at the amino group, indicating the unusually basicity of the amino group (see Figure 5.9). Comparing with adenine protonation, the proton adds at N(1), at the six membered ring instead of at the five membered ring. The protonated adenine form is then stabilized by the resonance hybrids (see Chapter 4, Figure 4.17), which means that the π electrons are delocalized over the entire ring.

At high pH, deprotonation of guanine takes place at N(1) six membered ring with an additional negative charge and only under this condition we could obtain the adsorption behavior similar to adenine. Possibly the energy of the π*-orbital is influenced in a way that a negative charge at the six membered ring leads to an increase/decrease of the energy of the orbital rather than the protonation of the five membered ring.

5.3 COADSORPTION OF BROMOURACIL, ADENINE AND GUANINE ON AU(111)

The mutagenic base bromouracil (BrU) is an analogue of thymine and as such is incorporated at about the same rate by DNA polymerase and binds with adenine. Several studies have shown the possibility of forming stable DNA structures with these nonstandard base pairs without large structural alterations [102,111,112]. However, bromouracil has a much greater tendency to mispair with guanine than thymine [113].

In the past, it was believed that the presence of a halogen substituent at the five position was probably the main cause of shifting the keto-enol equilibrium towards to enol, suggesting that the presence of the rare tautomer could be an explanation for the mutagenic properties of BrU. Recently, theoretic investigations about uracil and its derivatives suggested that the presence of the substituent at the five position does not increase the presence of enol form [102].

Based on the different hypothesis suggesting the mutagenic effect at DNA and RNA molecules, we wanted to investigate the issue on the interaction of bromouracil with adenine, as well as with guanine.

5.3.1 COADSORPTION OF BROMOURACIL AND ADENINE

The behavior of bromouracil at pH = 2 is very similar to thymine, i.e., at negative potential both molecules are physisorbed on the surface and chemisorbed at positive potentials. In contrast, adenine forms a charge-transfer complex with their π^*-orbital and d-orbital of gold at negative potential and the physisorbed film is formed at positive potentials. Since thymine and bromouracil has almost the same adsoption behavior (except for the film dissolution) and only the interaction between bromouracil and adenine is not so strong than that observed between thymine and adenine, we tried to found an electrochemical way on acidic solution for the strength on interaction between the two bases.

It is known that halogen substitute at the five position decreases the solubility of the base. Therefore, the maximum bromouracil concentration used at this work was 5 mM.

In Figure 5.15, the adsorption behavior for the mixture adenine/bromouracil is shown. Starting from a bromouracil/adenine concentration of 5 / 0.0001 mM the needle peaks indicating the first order phase transition and subsequent formation of the physisorbed film and the transition region from II→III are clearly seen. Only a small change of these peaks is observed when compared with the pure bromouracil solution (Figure 5.15 (a)). This can be attributed to the presence of a small adenine concentration at the electrode surface. Raising the bromouracil/adenine concentration of 5mM / 0.001 mM, the formation of physisorbed BrU film becomes less stable. Increasing further the adenine concentration, the transition region of bromouracil from physisorbed to chemisorbed phase is suppressed. The dissolution of bromouracil is accompanied by the formation of adenine chemisorbed, film indicating that both molecules are adsorbed on the surface (Figure 5.15 (d)). It seems that at higher adenine concentrations, the formation of physisorbed film of bromouracil is prevented.
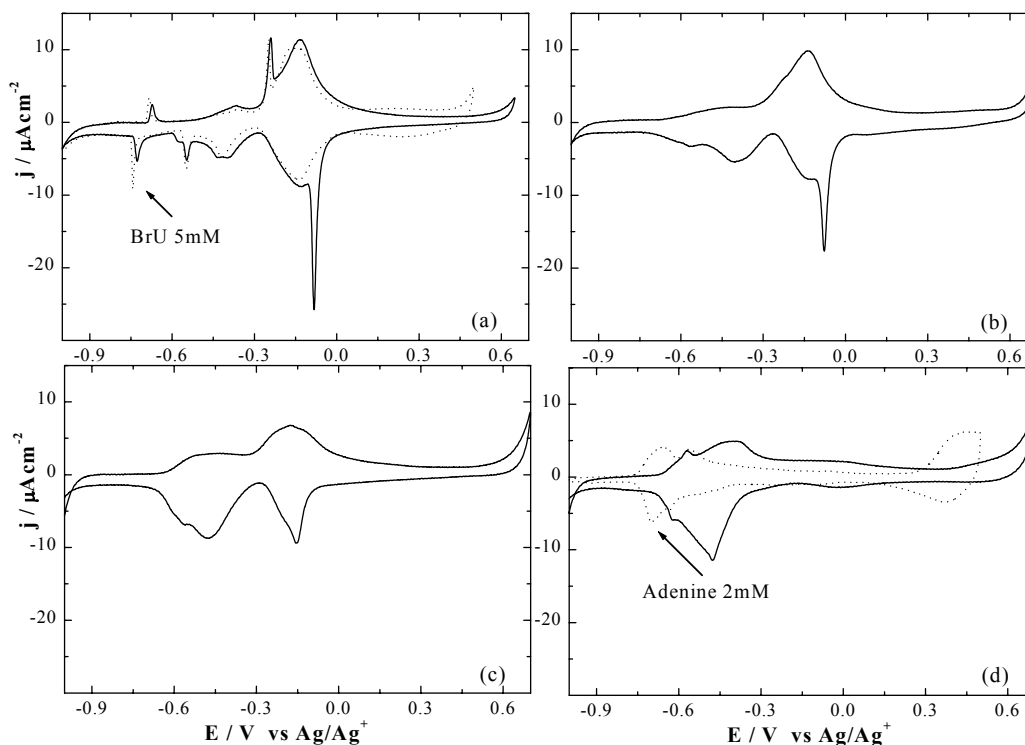
Figure 5.15: Cyclic voltammograms of bromouracil 5mM + adenine 0.0001 mM (a), bromouracil 5 mM + adenine 0.001 mM (b), bromouracil 5 mM + adenine 0.01 mM (c), and bromouracil 5 mM + adenine 0.1 mM (d). Supporting electrolyte: 0.1 M NaClO$_4$ (pH = 2). Sweep rate: v = 50 mV/s and T = 20 ^{o}C.

It is interesting to note that the lower is the bromouracil surface concentration; the more is the dissolution of the chemisorbed phase takes place in only one step.

If we add now small amounts of bromouracil in a solution of fixed concentration of adenine (of 2 mM) as presented in Figure 5.16, we observed that the adenine peaks does not change the shape in a significant way. It means that the electrode surface is still highly covered by adenine molecules and even an adenine / bromouracil ratio of 1 is not enough to change this strong adsorption.
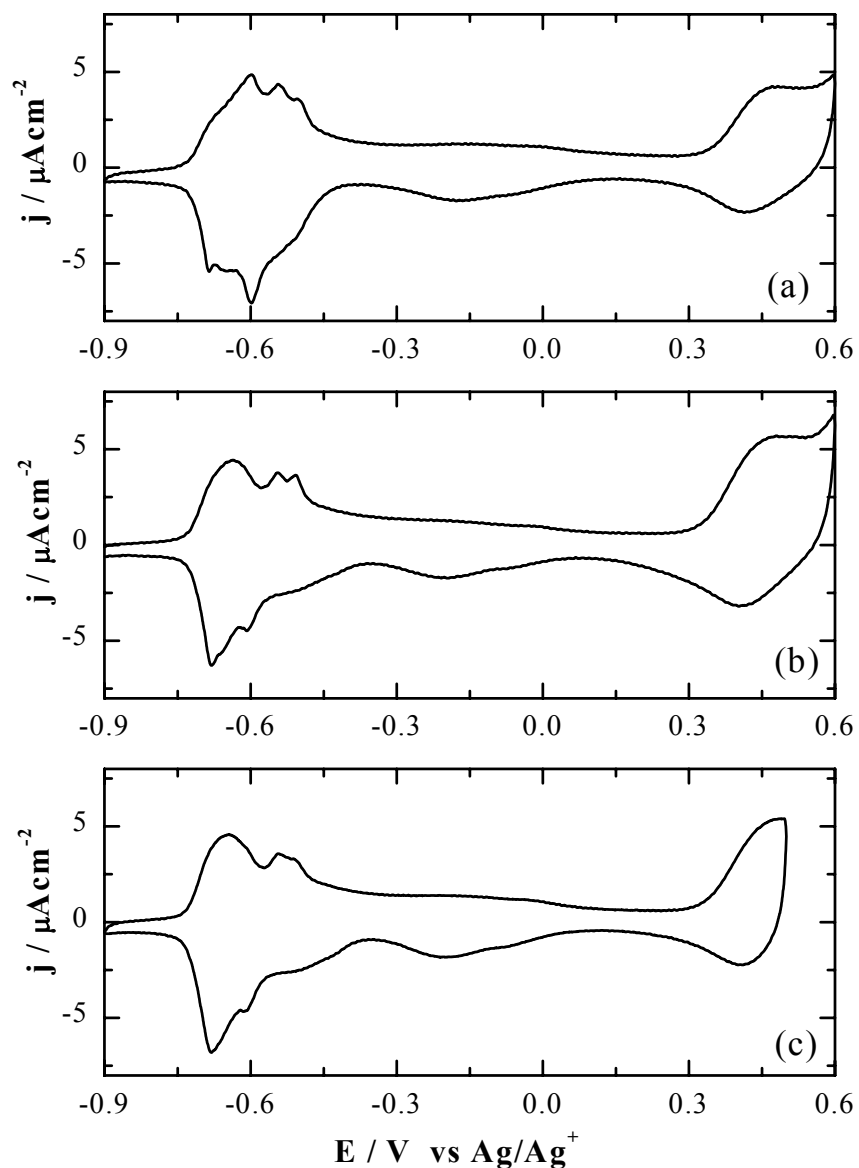
Figure 5.16: Cyclic voltammograms of adenine 2 mM + bromouracil 1 mM (a), adenine 2 mM + bromouracil 0.1 mM (b), adenine 2 mM + bromouracil 0.01 mM (c). Supporting electrolyte: 0.1 M NaClO$_4$ (pH = 2). Sweep rate: v = 50 mV/s and T = 20 $^{\circ}$C.

Unfortunately, we could not find any indication for a real appreciable interaction between bromouracil and adenine. The reasons can be: (a) the lower interaction between bromouracil and adenine as suggested by calculations [102] and (b) the structure of bromouracil at negative potentials, forming a very stable film, which is stabilized by hydrogen bonding (see Figure 5.6).

5.3.2 COADSORPTION OF BROMOURACIL AND GUANINE

Once it has been shown by calculations [102] that BrU interact better with guanine than with adenine, we ask now whether, owing to this strong interaction, the structure of BrU at the surface change upon addition of guanine. It is important to remember that at pH = 2 guanine behaves like a pyrimidine, i.e., it forms a physisorbed film at negative potentials and chemisorbed film at positive potentials. In contrast, however, no charge-transfer complex is formed at negative potentials, as observed with adenine.

For a bromouracil / guanine concentration of 5 / 0.0005 mM at pH value of 2, the phase transition peaks indicating the formation/dissolution of the physisorbed film and the transition region from II→III are present. Comparing the cyclic voltammograms in presence of guanine with pure bromouracil, only a small change can be seen (Figure 5.17 (a). Increasing the guanine concentration, the needle peaks change towards more positive potentials (Figure 5.15 (b)), indicating that in at higher concentration of guanine the stability of physisorbed condensed bromouracil decreases. Raising further the guanine concentration, the bromouracil condensed physisorbed film disappears, similarly to that observed for BrU-adenine.
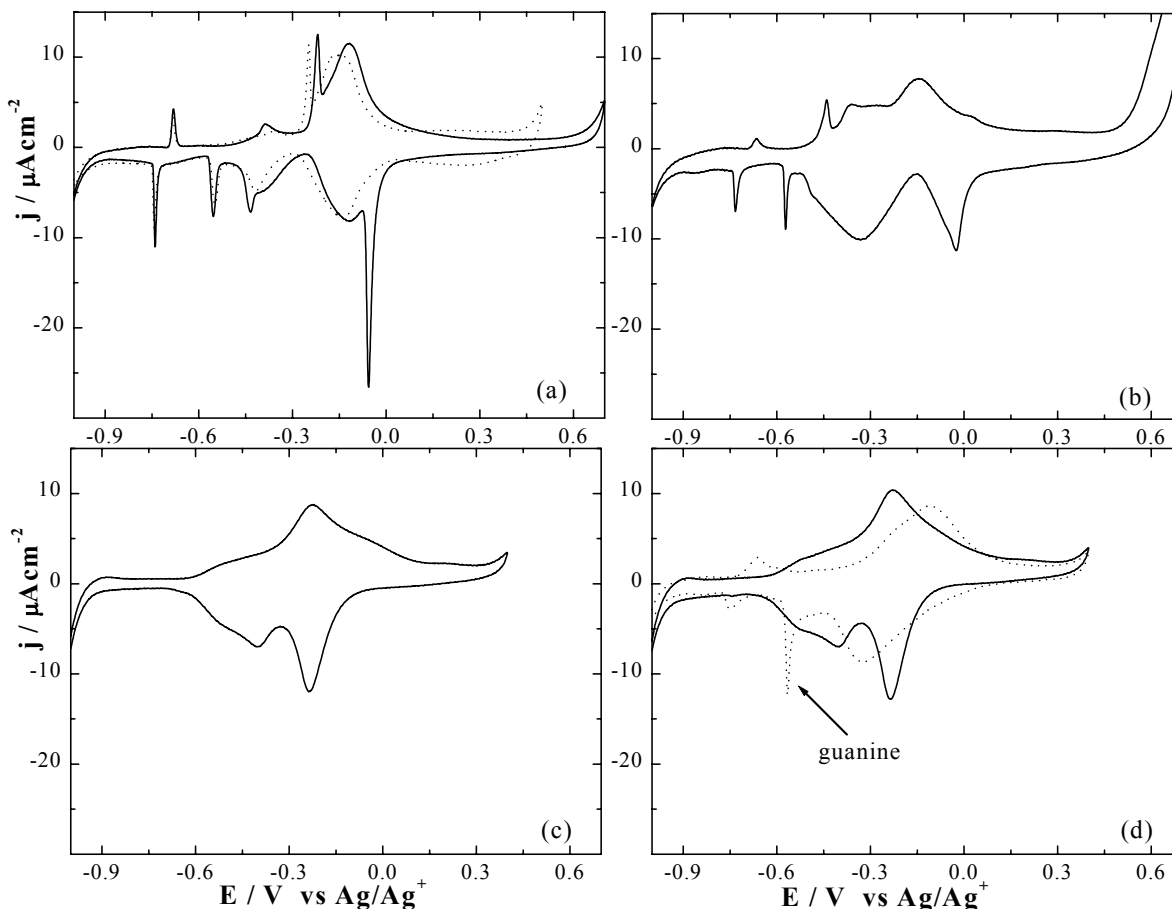
Figure 5.17: Cyclic voltammograms of bromouracil 5 mM + guanine 0.0005 mM (a), bromouracil 5 mM + guanine 0.005 mM (b), bromouracil 5 mM + guanine 0.05 mM (c), and bromouracil 5 mM + guanine 0.1 mM (d). Supporting electrolyte: 0.1 M NaClO$_4$ (pH = 2). Sweep rate: v = 50 mV/s and T = 10 ^{o}C.

Comparing guanine adsorption with bromouracil at pH = 8, we know that guanine is probably chemisorbed at negative potentials while bromouracil is randomly adsorbed or physisorbed at the same region. Due to this different adsorption state at the same potential region, we investigated the adsorption behavior of a mixture at pH = 8.

By adding a small amount of guanine to a fixed bromouracil concentration the cyclic voltammogram do not modify markedly (Figure 5.18). The CV shows the same shape as for the pure bromouracil solution. Increasing further the guanine concentration the shape of the CV is still close to that of bromouracil, but the intensity of the peaks becomes weaker (Figure 5.18 (b)). An increase of guanine concentration to 0.05 mM or higher changes drastically the shape of the CV. The peaks Br$_2$/Br'$_2$ and Br$_3$/Br'$_3$ are lowered and new peaks G$_1$/G'$_1$ appeared due to the guanine adsorption. It means that only a small concentration of guanine is

enough to repel bromouracil from the electrode surface, indicating the strongest adsorption of guanine in comparison to the other bases. Again, we have no indication of interaction between adsorbed bromouracil-guanine at pH = 8.
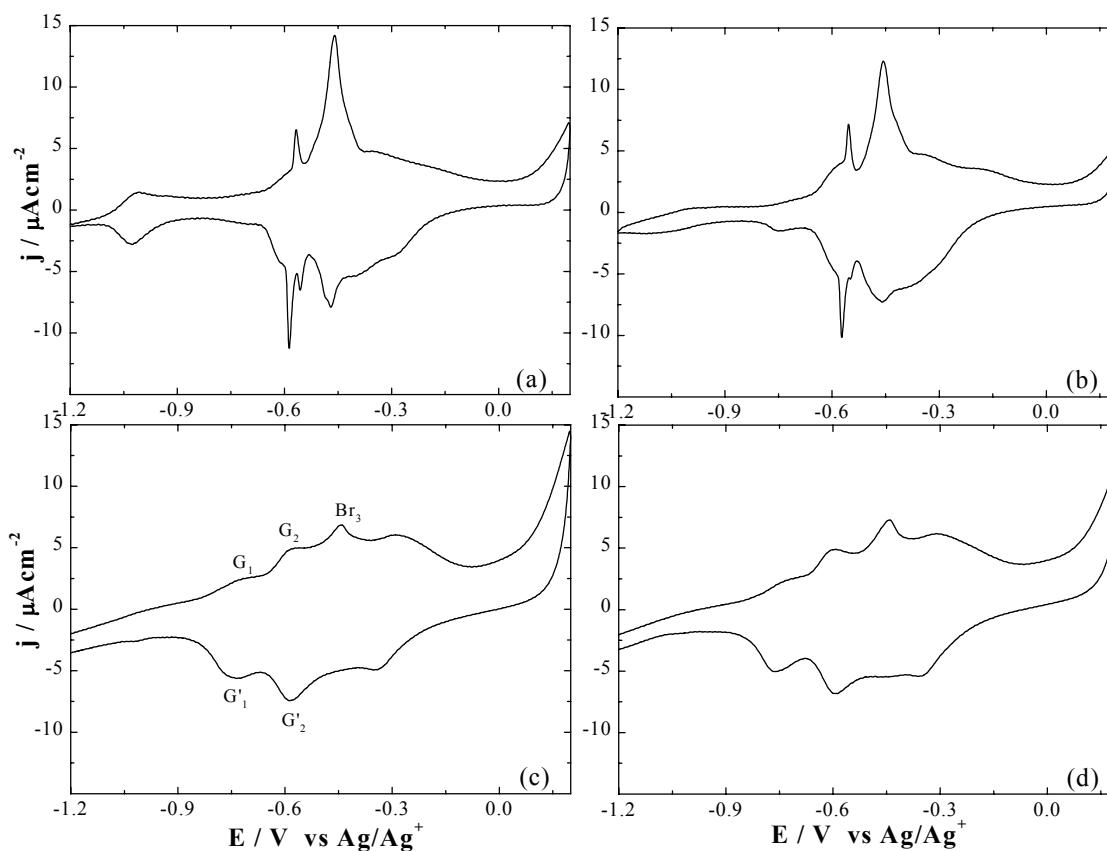

Figure 5.18: Cyclic voltammograms of bromouracil 5 mM + guanine 0.0001 mM (a), bromouracil 5 mM + guanine 0.005 mM (b), bromouracil 5 mM + guanine 0.05 mM (c), and bromouracil 5 mM + guanine 0.1 mM (d). Supporting electrolyte: 0.1 M NaClO$_4$ (pH = 8). Sweep rate: v = 50 mV/s. T = 10 $^{\circ}$C.

The absence of interaction between bromouracil and guanine can be rationalized in terms of: (a) at pH = 2: the high stability of bromouracil structure, which could not be disturbed by adding guanine in the solution (as for BrU-adenine); and/or (b) at negative potentials due to the non-planarity of the amino group in the guanine anion. In consideration (b) (the N(1) deprotonated form), the absence of hydrogen-hydrogen repulsion caused by the N(1)H results in a similar dihedral angles for both amino hydrogen [110].

5.4 COADSORPTION OF THYMINE AND GUANINE ON AU(111)

Guanine is a purine as adenine, and as it was already seen that its behavior change drastically depending on the solution pH (Figure 5.10 for pH = 2 and Figure 5.11 for pH = 6). One should remember that in acidic media guanine behaves as a pyrimidine, i.e., at negative potentials it forms a physisorbed film and a chemisorbed film is formed at positive potentials, similar to that for thymine and uracil. As a consequence, it does not make sense to investigate the coadsorption of thymine-guanine at pH = 2, probably, the results will be same as the obtained for thymine-uracil (see Chapter 4, Section 4.5) and bromouracil-guanine (Section 5.3.2).

As we assumed a similar behavior for guanine at pH = 8 and adenine at pH = 2, we asked if is it possible to obtain the same interaction between thymine-guanine at pH = 8 as was obtained for thymine-adenine at pH = 2. Figure 5.19 shows the CV of guanine and thymine at pH = 8. Under these conditions, the chemisorbed film of guanine takes place at the same region where the physisorbed condensed film of thymine is formed (exactly the same conditions obtained for T-A at pH = 2).
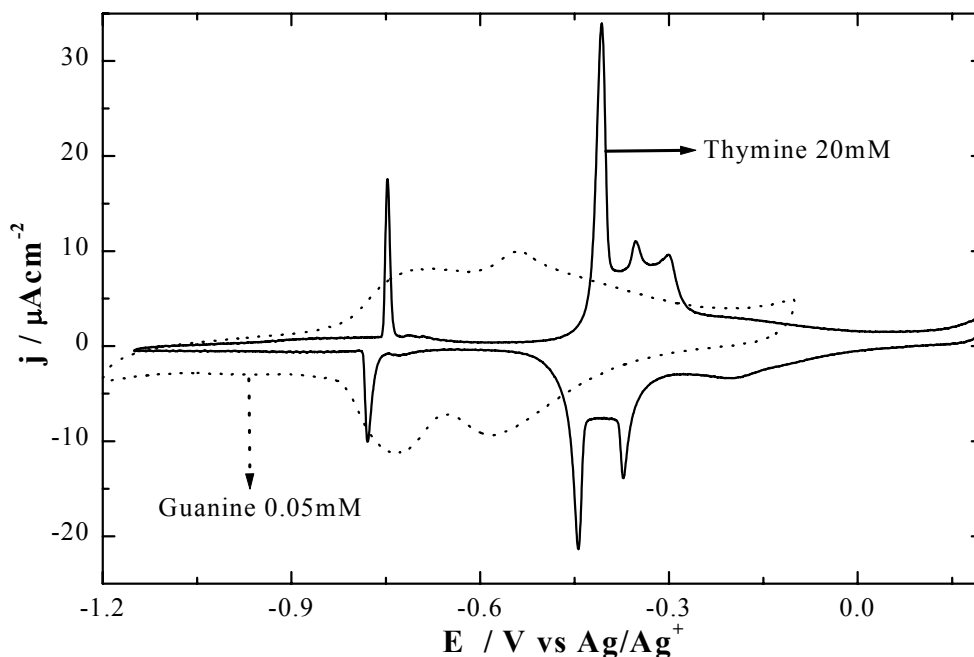
Figure 5.19: Cyclic voltammograms of thymine 20 mM (solid line) and guanine 0.05 mM (dotted line). Supporting electrolyte: 0.1 M $NaClO_4$ (pH = 8). Sweep rate: v = 50 mV/s. T = 20 ^{o}C.

Considering the poor solubility of guanine, the used concentration range was between 0.05 mM and 0.005 mM. Adding a high guanine concentration in a thymine solution (Figure 5.20), the phase-transition peak disappeared. Instead of the peak T_2/T'_2 and T_3/T'_3 only a small wave appeared. On the other hand, the shape of the CV resembles that of guanine, but a carefully analyses on the cathodic peaks revels a change of the negative peaks to more positive potentials for the solution containing both thymine and guanine. Decreasing the guanine concentration to 0.03 mM in a fixed thymine concentration of 20mM, the phase transition peak in the anodic scan indicates the formation of the thymine physisorbed film.
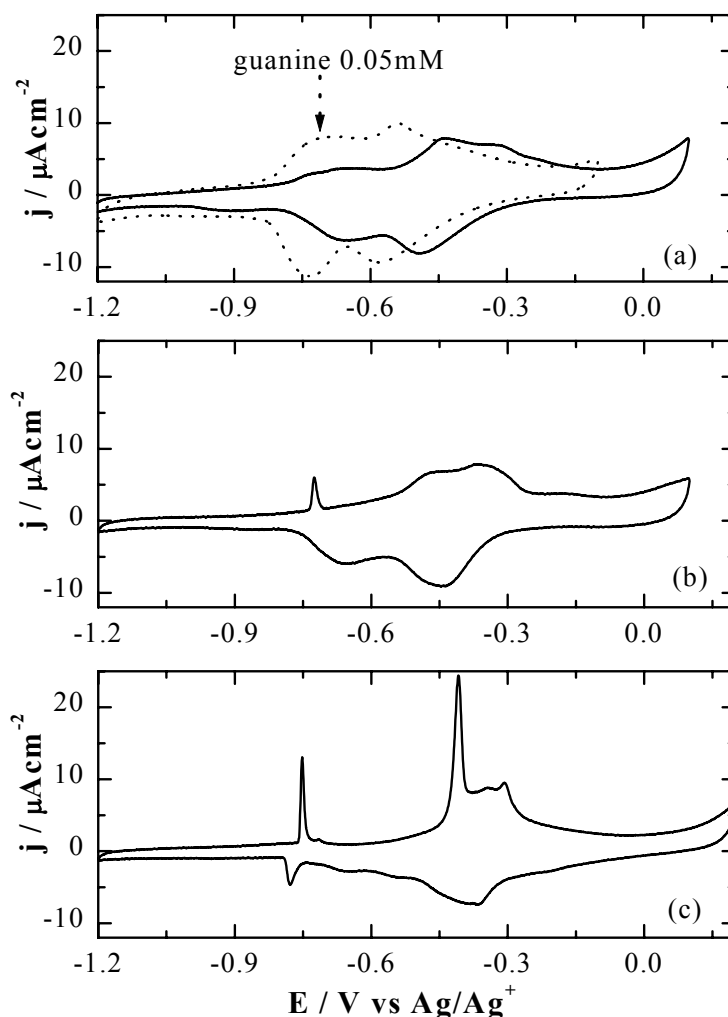
Figure 5.20: Cyclic voltammograms of thymine 20 mM + guanine 0.05 mM (a), thymine 20 mM + guanine 0.03 mM (b), and thymine 20 mM + guanine 0.005 mM (c). Supporting electrolyte: 0.1 M NaClO$_4$ (pH = 8). Sweep rate: v = 50 mV/s. T = 10 ^{o}C.

The peaks T$_3$/T'$_3$ shifts towards more positive potentials, indicating that the transition region from II→IV (region III for thymine adsorption, Figure 4.3, Chapter 4) is more stable due to the higher surface concentration of thymine. Lowering further the guanine concentration, the peak pairs T$_1$/T'$_1$, T$_2$ and T$_3$ are clearly seen. It means that at a thymine / guanine ratio of 40000 the thymine physisorbed and chemisorbed film are formed. Interesting is to note that the peaks T'$_2$ and T'$_3$ in the cathodic scan are not present, even when the guanine surface concentration is very low (Figure 4.3, Chapter 4). Fixing now the guanine concentration at 0.05 mM at pH = 8 and adding thymine to the solution, the CV resembles guanine, but the cathodic peaks G'$_1$/G'$_2$ changed towards more positive potentials when compared with guanine alone (Figure 5.21). Increasing the thymine concentration the shape

of the CV also changes, evidencing the appearance of additional peaks, which indicates the presence of thymine on the electrode surface. This is indicated by the small peak at negative scan around –0.85 V and by the anodic peak around –0.30 V, regarding the dissolution of the physisorbed condensed film of thymine and to the formation of the chemisorbed film at positive potentials.
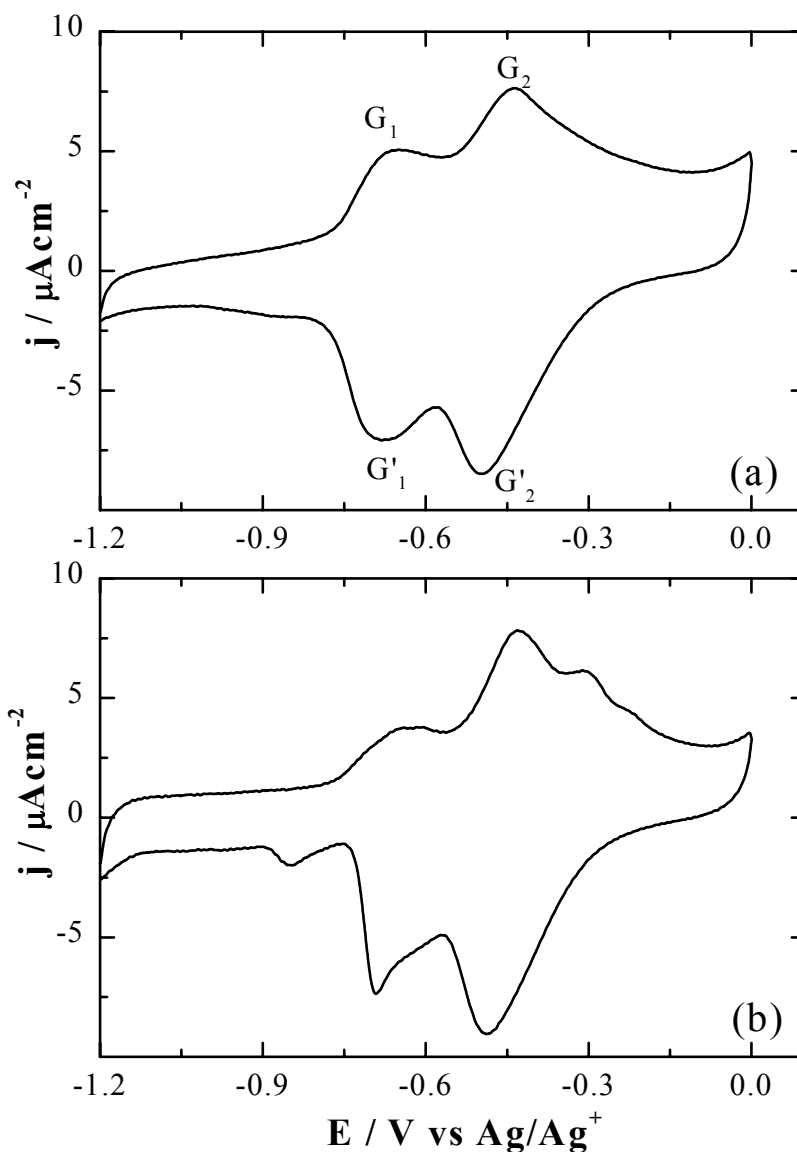

Figure 5.21: Cyclic voltammograms of guanine 0.05 mM + thymine 5 mM (a), and guanine 0.05 mM + thymine 10 mM (b). Supporting electrolyte: 0.1 M NaClO$_4$ (pH = 8). Sweep rate: v = 50 mV/s. T = 20 ^{o}C.

5.5 SUMMARY

In this chapter the adsorption behavior of bromouracil, adenine and guanine, as well as the coadsorption between these nitrogen bases was presented. The adsorption behavior of bromouracil is very similar to thymine in the pH values of 2 and 8, but guanine (purine) has a completely different and unexpected adsorption when compared with the purine base adenine. For the coadsorption kinetics between bromouracil–adenine and bromouracil-guanine no indication of interaction was found.