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Surface enhanced Raman spectroscopy study of the potential dependence of thymine on silver electrodes

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Abstract The potential-induced changes in thymine coordination on polycrystalline silver electrodes are studied by surface enhanced Raman spectroscopy (SERS) for potentials positive to the potential of zero charge up to the end of the double layer range. Two distinct sets of spectra could be obtained in the range of potentials studied. Both states correspond to chemisorbed phases of thymine on silver, where a distinct heteroatom is deemed responsible for the bond with the surface. At less positive potentials, one of the ring oxygen atoms is responsible for the chemical bond and the molecule assumes a tilted position. At more positive potentials, one of the ring nitrogen atoms, possibly deprotonated, establishes a new bond with the surface, aligning the molecule's axis closer to the surface normal.

Keywords Adsorption · DNA bases · Phase transition · Silver surface

Introduction

In this work we show that thymine chemisorbed on a silver electrode may assume two distinct geometries. Surface enhanced Raman spectroscopy (SERS) was employed to study this system in situ.

Dedicated to Prof. Wolf Vielstich on the occasion of his 80th birthday in recognition of his numerous contributions to interfacial electrochemistry

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The investigation of the adsorption behavior of thymine on silver single-crystal electrodes started with the work of Popov et al. [1]. In this initial communication, the changes of the Ag(111) electrode capacity were investigated as a function of the applied potential in solutions containing thymine at several concentrations. The interpretation given to these results pointed towards a single-step adsorption behavior, without indication of any two-dimensional molecular condensation. In an associated work, Popov et al. [2] showed that, as opposed to silver, thymine does condense on the surface of Cd(0001).

More recently, Hölzle et al. [3] established that, in fact, thymine does present a two-dimensional condensation on well-prepared Ag(111) and Ag(100) electrodes. They also noticed that the voltammetric behavior of thymine on these electrodes resembles the characteristic curves found for the corresponding single crystalline faces of gold. The potential range corresponding to the electric double layer of this system is usually divided into four adsorption states [4]. The nature of the adsorption state at the upper limit of this range was identified with chemisorbed molecules, forming a chemisorbed two-dimensional phase, showing high stability to temperature changes. An interesting feature in the data presented in that work was a pair of weak peaks around -0.4 V/SCE. The authors did not present a positive identification for them, but speculated about a possible phase transition. In a later work, Hölzle et al. [5] 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. [6] found, in a SNIFTIRS study, that thymine indeed chemisorbs to a gold surface through a nitrogen atom.

A picture that emerges from these works, and several others [7, 8, 9, 10, 11] on the same subject, points

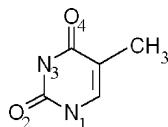


Fig. 1 Structural formula of thymine

towards a stable chemisorbed thymine layer. This layer would be bound to the metallic surface (gold or silver) through one of the nitrogen atoms, probably N₃ in the numbering scheme illustrated in Fig. 1. The phase transition suggested by Hölzle et al. [4] does not seem to be the subject, to our knowledge, of further inquiries into this system.

To investigate this subject we have decided to use SERS as a probe of the adsorbate molecular structure. The use of this technique to study thymine is not new. Koglin et al. [12] and Otto et al. [13] have already obtained SER spectra of thymine adsorbed on solid silver electrodes. Both studies focused on the adsorption of thymine from neutral solutions at potentials close to or slightly higher than the point of zero charge of the electrode. Otto et al. [13] commented in their communication that in excursions into more positive potentials, a change in the spectra was observed. To our knowledge, this observation was not pursued further. In another work, Koglin et al. [14] presented SERS results for thymine adsorbed on colloidal silver. A common point in all these publications is the complexity and variability of the results. Recently, Aroca and Bujalski [15] published a detailed study where these results were reviewed and compared with Aroca's own results. Their comments highlight the fact that thymine is a molecule with a very complex band assignment. The surface preparation, the environment, and even wavelength of the incident radiation may lead to a dissimilar spectrum. Nevertheless, a general trend, pointed out by Aroca and Bujalski [15], is that there are at least four possibilities for a chemical bond between the surface and the molecule. This bond may be formed between the surface and one of the two nitrogen atoms or one of the two available oxygen atoms and the surface. These two possibilities correspond to chemisorption and the wavenumber of this bond is located in the neighborhood of 210–230 cm⁻¹.

We will show that, depending on the surface potential, one or another of these atoms is indeed used. To perform the band assignment, we shall use primarily the results of Aroca and Bujalski [15]. To clarify some band assignments, the work of Aida et al. [16] and Aamouche et al. [17] will also be considered.

Experimental

Thymine (Fluka, puriss.) was used as received. The solution for in situ measurements was prepared from "Milli-Q" water, 0.05 M HClO₄ (Merck, suprapure), and 0.006 M thymine. For the Raman spectrum, a KBr pellet with a small amount of thymine was prepared. The SERS substrate was a roughened silver electrode with

ca. 0.5 cm² of geometrical area. All the potentials are referred to an Ag/AgCl/KCl(sat.) reference electrode. A platinum wire was used as a counter electrode.

The Raman and SERS spectra were acquired with a Renishaw Raman System 3000 equipped with an Olympus microscope (BTH2) with an 80× objective to focus the laser beam on the sample. The spectra were excited by the 632.8-nm line from an air-cooled He-Ne laser (Spectra Physics). The laser power measured after the microscope objective was ca. 50 mW. In all the experiments the silver electrode was activated by performing successive oxidation–reduction cycles in the working solution, from –0.7 V up to 0.7 V. The limits used were determined by the threshold of hydrogen evolution at the negative end and surface oxidation at the positive one. Prior to this electrochemical procedure, the silver electrode was gently roughened with sandpaper and cleaned in an ultrasonic bath. The electrochemical system used was a PAR 263 potentiostat-galvanostat from EG&G.

Results

To facilitate the assignment and the description of the structure of the adsorbed thymine, a molecular formula of thymine with some numbered atoms is shown in Fig. 1. In Fig. 2 we present a Raman spectrum of solid thymine. This spectrum bears close resemblance to the ones presented by Aroca and Bujalski [15], Koglin et al. [12, 14], and Otto et al. [13]. There are several discrepancies, however, and these are related to surface preparation and environmental conditions. For a complete discussion of these differences, reference should be made to the work of Aroca and Bujalski [15].

The SER spectra of adsorbed thymine molecules on the silver electrode at different electrode potentials highlight the effect of the applied potential on the SERS behavior of the adsorbed thymine (see Fig. 3). The spectra for adsorbed thymine on the electrode polarized at 0.5 V and 0.6 V are similar to that reported by Otto et al. [13] and by Koglin et al. [12] for the Ag electrode close to the potential of zero charge (p.z.c.). The p.z.c. for

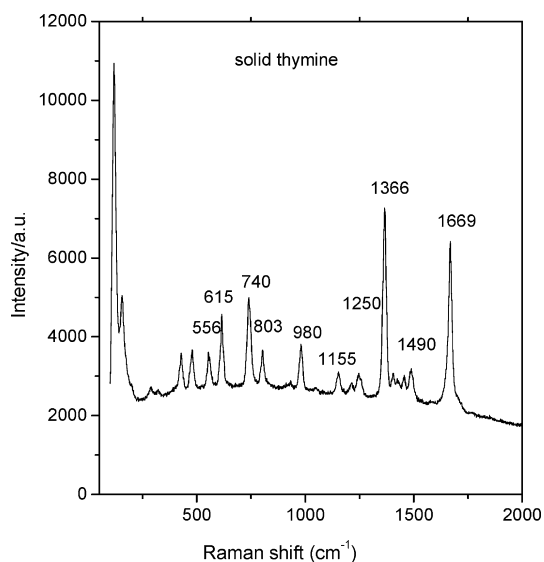


Fig. 2 Normal Raman spectrum of solid thymine recorded at $\lambda = 632.8$ nm

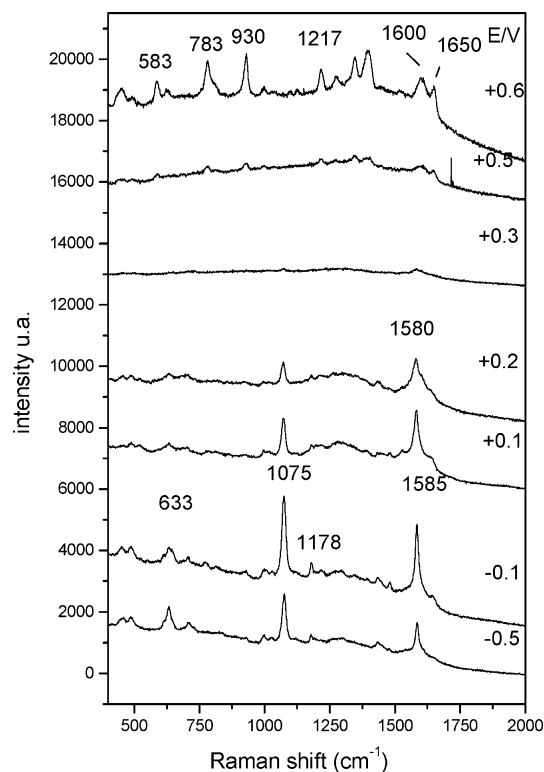


Fig. 3 Representative SER spectra of thymine adsorbed on silver in a 0.05 M HClO₄ + 10⁻³ M thymine solution as a function of potential; $\lambda = 632.8$ nm

polycrystalline silver in acid and alkaline media has been measured to be -0.76 V vs. Ag/AgCl [18]; thus, in our case, we are always positive to the p.z.c. The acidity of the solution precludes any study for potentials at or negative to the p.z.c., since hydrogen evolution takes place at -0.4 V vs. Ag/AgCl (sat. KCl). The spectrum obtained below $+0.1$ V is quite different, showing intensification of the bands at 1075 cm⁻¹ (which is absent in the spectrum at 0.6 V) and 1585 cm⁻¹ (compare with the corresponding band at 1595 cm⁻¹, that is of similar intensity in the spectra for potentials below 0.3 V).

Another feature worthy of note is a couple of strong and broad bands in the lower limit of the spectrum (Fig. 4). While the lower frequency band does not show any displacement with the applied potential, the higher frequency one does. At more negative potentials, this band has its center positioned around 216 cm⁻¹, and at positive potentials it is found at 226 cm⁻¹. Taking into account the assignment proposed by Aroca and Bujalski [15], this displacement is rather suggestive, since the first band is assigned to an oxygen-bonded adsorbed thymine, while the second one is expected for a molecule that is nitrogen-bonded to the surface [15]. The thymine has two carbonyl groups and two ring nitrogen atoms from where the bonding can occur. Therefore it is necessary to analyze in more detail all the bands to decide which nitrogen and oxygen atoms are used in the bonding to the silver surface. Bonding using the nitrogen atoms entails deprotonation of one of the ring nitrogens.

There is one distinct potential dependent band around 783 cm⁻¹ worth analyzing in more detail. The band at 783 cm⁻¹ is assigned to the ring-breathing mode (the corresponding band in solid thymine would be 740 cm⁻¹). This band is present in all publications detailed above as a prominent band. In the present study, this band is visible mainly at more positive potentials and is a band of medium intensity. At -0.5 V the band intensity practically vanishes. This vibrational mode is entirely in the molecular plane, and an increase in its intensity may be indicative of an alignment of the molecular axis with the normal to the surface. Therefore we assume that at -0.5 V the molecule must be flat or strongly tilted on the surface to account for the absence of the in-plane ring-breathing band. However, the strong intensification of the band at 216 cm⁻¹ suggests that the molecule binds to the surface using one oxygen atom. Therefore the most plausible orientation is a molecule tilted on the surface. At -0.1 V the band starts to be visible and the intensity increases up to $+0.6$ V. As pointed out above, this means that the molecule must align the molecular axis to the surface progressively as the potential is made more positive.

Another band with an interesting behavior is the one found around 1075 cm⁻¹, as shown in Fig. 3. This band occurs for the adsorbed thymine on silver only for more negative potentials (closer to the p.z.c.), presenting a remarkable intensification. Its intensity decreases until vanishing at $+0.5$ V. For further discussion of the possible molecular orientation, it is important to establish the assignment of this band.

Otto et al. [13] did not detect this band in their spectra for thymine adsorbed on silver electrodes at potentials close to the p.z.c. However, Aroca and Bujalski [15] found a similar band at 1066 cm⁻¹ for thymine adsorbed on silver islands film. This band was identified as the band at 1049 cm⁻¹ of solid thymine and has been assigned to a twist mode of the methyl group. Aamouche et al. [17] could not identify this band for the solid sample, but did find it for thymine in solution at 1063 cm⁻¹, and also assigned this mode to an asymmetrical rock of the methyl group. Aida et al. [16] found bands at 1091 cm⁻¹ and 1027 cm⁻¹, which could be identified with this band. In both cases, they assigned a methyl group rocking mode as responsible for the band. Therefore, it is clear that the assignments presented identify this band with some mode of the methyl group.

The intensification of this band for potentials lower than 0.1 V indicates that the methyl group is closer to the surface at these more negative potentials. The absence of this band for more positive potentials indicates, on the other hand, that the methyl group is moved away from the surface. This assignment puts forward the idea that, for potentials closer to the p.z.c., the molecule must be bonded through the oxygen closer to the methyl group, i.e. O₄, at more negative potentials.

It is also important to discuss the behavior of the 1355 cm⁻¹ band, that can also be assigned to a methyl group vibrational mode, i.e. the symmetric CH₃

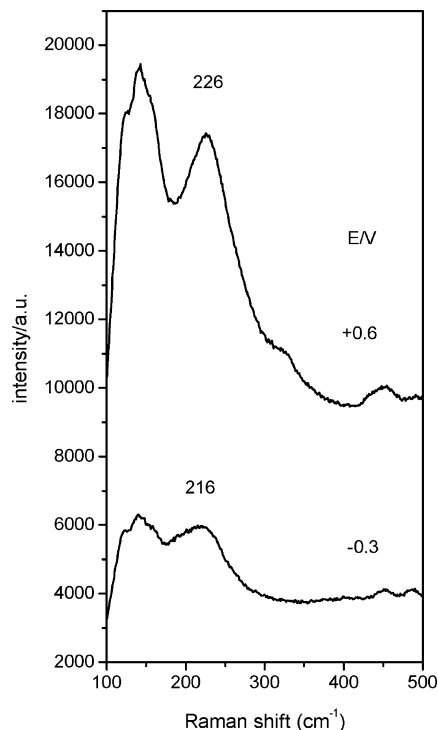


Fig. 4 SER spectra of thymine adsorbed on a rough silver electrode in a 0.05 M HClO₄ + 10⁻³ M thymine solution at the low wavenumber side of the spectrum; $\lambda = 632.8$ nm

deformation. While this mode is very strong in the solid state spectrum of thymine, it is visible only at +0.5 V and +0.6 V in the SER spectra, and vanishes close to the p.z.c. The different behavior observed for these two bands assigned to the CH₃ moiety in thymine can be understood in terms of their different orientations. The methyl group mode at 1075 cm⁻¹ has a considerable out-of-plane component, as opposed to the symmetric deformation mode at 1355 cm⁻¹. The symmetric stretching mode of CH₃ thus behaves like motions parallel to the thymine ring (like, for example, the ring-breathing mode at 783 cm⁻¹), while the 1075 cm⁻¹ mode is highly enhanced, due to its out-of-plane component together with the position of the CH₃ group close to the metallic surface.

A weak band at 1178 cm⁻¹ presents the same behavior as the previous one. Otto et al. [13] observed this band at 1084 cm⁻¹. In the study by Aroca and Bujalski [15], this band was found in the SER spectrum of thymine adsorbed on silver islands film at 1160 cm⁻¹ and they identified this band at 1156 cm⁻¹ for solid thymine. This band was assigned to torsion of the methyl group. The same interpretation was made by Aamouche et al. [17] for a band at 1161 cm⁻¹ (solid) and 1165 cm⁻¹ (solution). As the band at 1178 cm⁻¹ can be assigned to a methyl group mode, the same reasoning used for the previous band applies here.

In the same spectral range the band at 1217 cm⁻¹ presents opposite behavior, i.e. the band vanishes for more negative potentials and presents an increasing

intensification for more positive potentials. Aroca and Bujalski [15] and Otto et al. [13] assigned this band to a mixture of the methyl stretch and the ring vibration modes. This is a difficult assignment, but the accuracy of this choice can be ascertained by the fact that this band is also present with halogen derivatives of uracil [19]. In fact, molecules where the methyl group was substituted by halogens still presented the same strong band at 1214 cm⁻¹, showing that the main component of this mode is the ring stretch. Aamouche et al. [17] assign this band specifically to a stretch between the C₆ and N₁ bond, indicating that this vibrational mode is in the molecular plane of the molecule.

Taking into account the above assignment, it is clear that the molecule must be tilted on the surface for potentials closer to the p.z.c. and it aligns progressively with the surface normal for more positive potentials, as stressed above. The alignment of the molecule to the surface normal is followed by an increase in the surface-methyl group distance, as attested by the vanishing intensity of the band at 1075 cm⁻¹. This reorientation of the molecule requires the change from an O₄ bonding to a nitrogen bonding, as suggested by the change of 216 cm⁻¹ (surface-O bond) to 226 cm⁻¹ (surface-N bond). The question that arises now is to relate the bonding at more positive potentials to a specific ring nitrogen.

The bands at 1650 cm⁻¹, assigned to C₄=O₄ and C₅=C₆, and the band at 1585 cm⁻¹, assigned to C₂=O₂ stretching (see Fig. 3), can give an additional clue to the potential-dependent molecular orientation on the surface. The band at 1650 cm⁻¹ has its intensity increased for more positive applied potentials. On the other hand, the band at 1585 cm⁻¹ decreases in intensity with the applied potential until ca. +0.5 V, when suddenly it changes to 1595 cm⁻¹. At +0.5 and +0.6 V the 1650 cm⁻¹ and 1600 cm⁻¹ bands have similar intensities. This result is consistent with C=O₄ and C=O₂ at practically the same distance to the surface. So far, in the potential-dependent molecular orientation proposed, the distance between the carbonyl group at C₄ and the surface slightly increases when the applied electrode potential is made more positive. This picture is consistent with bonding through the nitrogen N₃ to the surface. The sudden shift of the 1585 cm⁻¹ band to higher energy is probably related to the deprotonation of the nitrogen N₃, with subsequent bonding to the surface.

Discussion

The most plausible molecular orientation, deduced from the results discussed above, is that thymine is bonded to the silver surface through oxygen O₄ and tilted for potentials closer to the p.z.c. This molecular orientation contrasts with that observed by Otto et al. [13] and by Koglin et al. [12], for neutral solutions, where they found bands typical for perpendicular orientation for potentials close to the p.z.c. This orientation has been

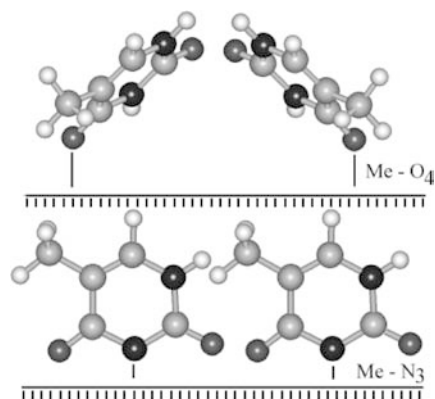


Fig. 5 Adsorption model for thymine on silver

observed upon changing the electrode potential positively in the present study. In a more complete model (see Fig. 5), the molecule, adsorbed through the oxygen atom, rotates and aligns to the surface normal, becoming more perpendicular on the surface as the potential is made more positive and changing from an oxygen-bonded to a nitrogen-bonded adsorbate.

Although not specifically stated by Otto et al. [13] and Koglin et al. [12], the thymine adsorbed from neutral solutions must present the same bonding as in acidic solutions at more positive potentials, since the spectra are very similar. The delay in the O- to N-bonding transition in the case of thymine is related to the fact that the nitrogens in thymine are sp^2 hybridized and bonding of the nitrogen to the surface is possible only upon deprotonation. Thus, to rationalize the different bonding at potentials closer to the p.z.c., we have to consider the dependence of proton transfer on the solution acidity, because even in neutral solutions thymine will be protonated in solution, since the pK_a for thymine is 10.07. Since the protonation on the ring nitrogen in thymine is a reversible reaction, it is clear that in acidic solution the proton transfer barrier must be higher than in neutral solutions. Therefore, the contact of thymine with the positively charged silver surface will induce proton transfer at different electrode potentials. The cyclic voltammogram of thymine adsorbed on a rough silver electrode displays a reversible redox peak at 0.20–0.25 V vs. Ag/AgCl (Fig. 6), revealing a charge transfer at the same potential where the transition of the SER spectra suggest a change in coordination.

Since proton transfer cannot take place close to the p.z.c. in acidic solutions, the alternative bonding is through the O_4 atom of thymine, with the methyl group close to the surface. The proximity of the methyl group to the positively charged surface is unexpected, since the methyl group of both 1- and 2-methylimidazole interacts with the surface of silver electrodes at potentials close to or negative to the p.z.c., as reported by Carter et al. [20], i.e. there is a clear repulsion of the methyl group by the positively charged surface. Actually there must be a balance between the charge density over the oxygen and the charge on the methyl group that stabilizes the

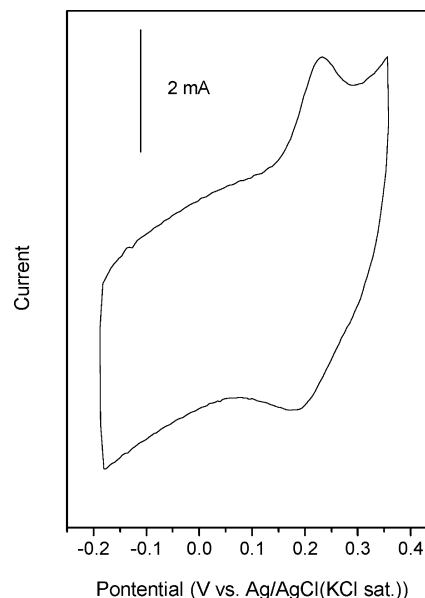


Fig. 6 Cyclic voltammetry of adsorbed thymine on a silver electrode in a 0.05 M $HClO_4 + 10^{-3}$ M thymine solution

bonding, keeping the methyl group close to the surface at potentials slightly positive to the p.z.c. This bonding prevails until the potential for proton transfer is reached. It is difficult to assign the transition to the increasing repulsion or to the energy to the proton transfer. The difference in behavior for the 1- and 2-methylimidazoles must rely on two factors that contribute to facilitate the change in the molecule position: the lack of oxygen to compensate for the repulsion of the methyl group with the surface and the absence of protonated nitrogen.

The adsorption of thymine at more positive potentials through the nitrogen N_3 and not nitrogen N_1 seems quite logical, since N_3 is closer to O_4 than N_1 . Moreover, N_3 is located between two oxygens that concentrate the negative charge and stabilize the bonding at this position on the positively charged surface.

Conclusions

The adsorption behavior of thymine in acidic solution on a silver electrode has been studied as a function of the applied potential. A two-state pattern could be observed and a model for the potential-induced molecular orientation on the surface is proposed. According to this model, an increase in the surface potential leads to a deprotonation of the molecule followed by a rotation of the molecule towards a position where its molecular axis is aligned with the surface normal. The response of several SER band intensifications has been carefully analyzed and lends support to the presented model.

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