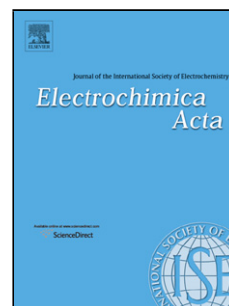


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In situ Fourier transform infrared reflection absorption spectroscopy study of adenine adsorption on gold electrodes in basic media.

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SUMMARY

In situ *Fourier transform infrared reflection absorption spectroscopy (FT-IRRAS)* has been used in the external (SNIFTIRS method) and the internal (ATR-SEIRAS) reflection configurations to determine the pH influence, in the neutral and basic range, on the adsorption of adenine on Au(111) and gold nanofilm electrodes from D₂O and H₂O solutions.

In D₂O solutions, the main adsorbate band around 1640 cm⁻¹, due to a ring stretching mode, shows different characteristics in the spectra collected at pH values at which the neutral and the basic adenine forms predominate in solution. The analysis of these differences, in comparison with the respective spectra of adenine in solution, permits us to conclude that both forms of adenine can adsorb chemically. The high sensitivity of the ATR-SEIRAS method has been used to analyze the contribution to the spectra of each form of adsorbed adenine as a function of the pH of the solution. The pK_{a2} obtained for the adsorbed species from this analysis is almost coincident with the pK_{a2} reported for adenine in solution, indicating that the coordination to the electrode and the second acid-base equilibrium involves different atoms of the adenine molecule. This result confirms the previously proposed adsorption model for adenine, implying the bonding of adenine to the electrode by the amine nitrogen (N₁₀) and either the ring nitrogens N₁ or N₇, while the second acid-base equilibrium of adenine involves the ring nitrogen N₉.

Comparison of the 3400-3600 cm⁻¹ region of the ATR-SEIRAS spectra of adenine obtained in H₂O solutions at different pH values, which corresponds to the characteristic -OH stretching mode of the interfacial water molecules, permits us to discard the co-adsorption of water molecules in neutral and basic media, contrary to the case of adenine adsorption from acid media.

Keywords: Gold electrodes; adenine adsorption; in situ FT-IRRAS; pKa determination.

1.-Introduction

Most of the processes occurring at bio-interfaces involve electrochemical phenomena. Because of that, it is interesting to study the behaviour of molecules of biological relevance at the electrode interface, which offers the possibility of controlling the electric field among other environmental parameters. In the case of DNA bases, there is some extra interest in the study of their adsorption at electrodes because of plausible applications in biosensors design, the fabrication of new bio-compatible materials and the development of new supramolecular organizations to be used as vectors for the cellular delivery of drugs with nucleotide structures.

We have previously studied the adsorption of adenine on gold single crystal and gold nanostructured electrodes, in neutral and acid media by means of different electrochemical, spectroscopic and microscopic techniques, [1-5]. The characterisation of the adsorption process by cyclic voltammetry and differential capacitance in neutral media on the low index gold single crystal electrodes [1] showed that the adsorption phenomena is strongly dependent not only on the crystallographic orientation, but also on the reconstructed or unreconstructed state of the surface. In fact, the adsorption of adenine on freshly flame annealed reconstructed gold surfaces is accomplished by the lifting of the surface reconstruction. The chronoamperometric study in neutral solutions of the thermodynamics of adenine adsorption on Au(111) electrodes concluded that chemisorption takes place with adenine acting as electron donor [1]. The maximum surface excess obtained from the thermodynamic data was consistent with a tilted orientation of the molecular plane relative to the electrode surface. The analysis of the frequency and electrode potential effects on the Electrochemical Impedance Spectroscopy measurements provided the kinetic parameters of adenine adsorption/desorption on Au(111) electrodes [2].

Fourier transform infrared reflection absorption spectroscopy (FT IRRAS) is a valuable tool in order to get molecular information from species adsorbed on solid electrodes interfaces. In situ IRRAS measurements can provide information about the nature of the adsorbates, their orientation relative to the electrode surface, the atoms involved in the interactions with the metal surface, the nature of the intermolecular interactions at the interface and the influence of the solvent. In a previous work, the adsorption of adenine on gold single crystal and gold thin-film electrodes was studied in neutral media by means of in situ FT-IRRAS measurements [3]. The spectroscopic results obtained were analysed on the basis of previously published data for the

adsorption of adenine on various substrates [11-33]. From this analysis it was proposed that, independently of the potential and of the crystallographic orientation of the electrode, adenine is coordinated to the gold electrode surface through the nitrogen of the amine group (N_{10}) and another nitrogen atom, probably the nitrogen (N_7) of the imidazol ring, with the molecular plane tilted relative to the electrode surface. This adsorption model involves a sp^3 hybridization for amine nitrogen N_{10} , as previously suggested for the adsorption of adenine on different substrates [33].

It is well established that the functions of biological compounds can be closely related to the acid dissociation equilibrium. In the case of DNA bases, the Watson-Crick interactions between complementary bases are more likely to occur when both bases are in their canonical form, and the presence of "unstable" tautomeric forms can yield to unpaired bases interactions [34-40]. On the other hand, the pKa values of biological molecules can change when located in organized environment, affecting their function [41-44].

- scheme 1 -

Adenine has two pKa values in aqueous media [45,46] as is shown in scheme 1. The first one, $pK_{a1}=4.2$, is related to the protonation of the pyrimidine nitrogen N_1 . The second, $pK_{a2}=9.8$ accounts for the loss of the proton in the imidazol nitrogen N_9 . Our previous in situ FT-IRRAS studies of adenine adsorption on gold electrodes in neutral and in acid media [3, 4] showed that only the neutral form of adenine (AdH_3) is chemisorbed on the electrode under these conditions, even at pH values as low as pH 1. Therefore, pK_{a1} value of chemisorbed adenine is much lower than pK_{a1} value of adenine in solution. However, the presence of protonated adenine (AdH_4^+) on the electrode surface is detected at low pH values and at potentials more negative than the onset of the chemisorption of adenine, because it yields surface active IR signals at the same frequencies than adenine in solution. The signals corresponding to AdH_4^+ on the surface disappear as the chemisorption of AdH_3 is favoured, by increasing either the potential or the adenine bulk concentration or the pH value. A physisorption state was proposed for AdH_4^+ . Recent in situ electrochemical STM experiments also suggest the weak adsorption of the protonated adenine [5].

On the other hand, the comparison of adenine adsorption in acid and in neutral media also reveals differences in the spectral signals corresponding to the solvent and to the anion of the supporting electrolyte [3,4], that indicate that the co-adsorption of these species with adenine takes place only in acid media.

The aim of this paper is to extend the spectroelectrochemical study of the adsorption of adenine on gold electrodes to pH values covering the pK_{a2} , in order to search for the adsorption characteristics of the anionic form of adenine (AdH_2^-) and to determine the pK_{a2} value at the interface. The possible coadsorption of the solvent is also investigated.

2.- Experimental.

Solutions were prepared either in ultra pure water from a Millipore Direct-Q purifier or in deuterium oxide (Sigma 99.99 %). Sodium hydroxide, perchloric acid and potassium perchlorate were from Merck Suprapur®. Adenine, from Sigma, was used without further purification. Stock solutions of adenine 10 mM were prepared in the same supporting electrolyte and spikes were added to the cell solution in order to get the desired working adenine concentration (0.01 mM, 0.1 mM or 1 mM). All the solutions were deaerated by bubbling argon (Air Liquide N50) during 30 min. prior to use.

Voltammetric experiments were performed with an Autolab PGstat 30 multipurpose electrochemical system, controlled by NOVA 1.7 software. The working electrode was a freshly flame annealed Au(111) single crystal electrode prepared following the procedure described by Clavillier [47]. A gold wire and a saturated mercury/mercurous sulphate electrode connected to the cell via a salt bridge were used as auxiliary and reference electrodes respectively. Solution's pH was measured with a PHM 64 pH meter from Radiometer and a Hamilton borosilicate membrane combined electrode. The pH readings on deuterium oxide solutions were corrected according to [48]. All the potentials are given vs. SCE.

FT-IRRAS spectra were obtained at a resolution of 4 cm^{-1} with a NICOLET 6700 spectrophotometer equipped with a narrow-band MCT-A detector cooled with liquid nitrogen, and a V-max II accessory for reflectance measurements. The spectra were collected either with p or s polarized radiation, selected with a ZnSe motorized polarizer, and are presented as the ratio $-\log(R/R_0)$ with R and R_0 being the reflectance spectra at the sample and reference conditions, respectively. Electrochemical control of the cell was made with a CHI 1100 A potentiostat from CH Instruments.

For external reflection experiments a thin layer configuration was used, with a CaF_2 prismatic IR window bevelled at 60° . The working electrode was an Au(111) single crystal. The same auxiliary and reference electrodes were used as in the cell

described above for voltammetric experiments. The external reflection spectra obtained were referenced to a desorption potential. In order to increase the signal to noise ratio, spectra calculated from sets of 50 interferograms were measured alternatively at the sample and at the reference potentials at least during 40 cycles, and the results were averaged, in the so called *Subtractively Normalized Interfacial Fourier Transform Infrared Spectroscopy* (SNIFTIRS) procedure.

Internal reflection spectra were obtained using the Kretschmann configuration of spectroelectrochemical cell: the cell window consisted in a silicon prism bevelled at 60°. The working electrode was a gold film deposited on to a face of the silicon prism by argon sputtering with a Leica EM SCD500 metalizer equipped with a quartz crystal microbalance to control the thickness and the speed of deposition (c.a. 25 nm and 0.01 nm s⁻¹). The three electrode cell was completed with the same reference electrode as above and a gold foil acting as auxiliary electrode. This configuration involves the total reflection of the IR beam at the inner face of the Si window. Only the evanescent wave penetrates a few hundred of nanometers into the solution, so the spectra can be more efficiently corrected from the solution contributions than with the external reflection set up. Moreover, the nanostructure of gold nanoparticles forming the film originates an enhancement of the radiation electric field and, therefore, increases the sensitivity of the obtained spectra. This kind of measurements is named *attenuated total reflection surface enhanced infrared absorption spectroscopy* (ATR-SEIRAS). The spectra measured can be referenced either to a potential at which adenine is desorbed or at the same potential but in the absence of adenine in the bulk solution. Every spectrum was obtained averaging 100 interferograms.

ATR infrared absorption spectra of adenine in solution were measured with a cell equipped with a ZnSe prismatic window bevelled a 45°. Each spectrum is obtained averaging 1000 interferograms, being referred to the single beam spectrum obtained for the corresponding solvent (either H₂O or D₂O) under the same conditions.

3.- Results and discussion

3.1- Cyclic voltammetry

Figure 1 show the stationary cyclic voltammograms obtained with an Au(111) electrode in solutions containing adenine at different pH values. Panels a) and b) also show the first scans, measured immediately after the freshly flame annealed electrode contacts the solution. These first scans show peaks that account for both the

adsorption of adenine and the lifting of the reconstruction of the electrode surface [1]. From second scan stationary behaviour is obtained. The time scale of the measurement is shorter than the time required for the electrochemical reconstruction [1], so the peaks that appear on the stationary scans are only due to the contribution of adenine adsorption/desorption. Electrochemical STM measurements [5], have revealed that the adsorption of adenine provokes the lifting of the reconstruction and stabilizes the unreconstructed surface.

The influence of pH is clearly manifest in Figure 1. The adsorption peak potentials shift towards lower values as the solution pH increases in the range from acid to neutral values, but remain constant as the pH change in the range neutral to highly basic values.

- Fig. 1-

The pH dependence of the voltammograms in acid and neutral media indicates deprotonation of adenine molecule in order to chemically interact with the metal. It was previously demonstrated [4. 5] that in acid media the cationic protonated adenine form, AdH_4^+ , can be physically adsorbed in a narrow potential region but the potential-induced chemical adsorption involves previous deprotonation, so the uncharged adenine form, AdH_3 , is the one that can coordinate the gold atoms on the surface. On the contrary, the pH independent peak potential obtained at pH values around pK_{a2} or higher, indicates that the anionic adenine form (AdH_2^-) can be chemically adsorbed on the electrode, as the neutral form does. The voltammograms also show that the kinetics of the adsorption process is pH dependent. At high pH values the voltammograms are almost reversible, contrary to the case of neutral or acid pH values. The kinetics of adenine adsorption in neutral or slightly basic media was studied by impedance measurements [2] and further analysis in acid and basic media are in progress. In this paper the differences in the adsorption characteristics of the neutral and basic adenine forms are pursued by spectroscopic data.

3.2.-Infrared spectra of adenine in solution.

For the sake of comparison, the infrared absorption spectra of adenine 10 mM solutions at two different pH values were obtained. The results in the range $1500\text{-}1800\text{ cm}^{-1}$, which shows the most intense and relevant bands for our study, are given in figure 2. The two main signals in this region have been assigned to the scissoring mode of the amino group (the one at higher wavenumbers) and to a skeletal ring stretching mode including $\text{C}_5\text{-C}_6$, $\text{C}_6\text{-N}_{10}$, $\text{C}_4\text{-C}_5$, $\text{C}_2\text{-N}_3$, $\text{N}_1\text{-C}_2$ vibrations [11]. To

overcome the interference of solvent caused by the –OH bending mode of H₂O at c.a. 1650 cm⁻¹, the spectra have also been obtained in D₂O media, that makes the –OD bending band shifts to c.a. 1200 cm⁻¹ [49].

- Fig 2-

The spectrum in D₂O neutral media of deuterated adenine, AdD₃, shows the main ring stretching mode at 1624 cm⁻¹. The band in basic D₂O media for the anionic form, AdD₂⁻, is red-shifted with respect to the signal for AdD₃ as previously reported for the sodium salt of adenine [49,50].

In H₂O solutions the bands also shift to lower wavenumbers as the pH value of the solution increases. However, the main bands overlap with the contribution from the bending mode of water molecules, so broader signals appear in the solutions at all the two pH values. Therefore, the spectra for characterisation of the adsorbed species at pH values around the pK_{a2} value, were collected using D₂O as the solvent.

3.3.-In situ FT-IRRAS of adsorbed adenine from neutral and basic D₂O solutions.

Some SNIFTIRS spectra of adenine adsorbed on Au(111) electrodes from neutral and basic D₂O solutions are shown in figure 3. These spectra were collected at a high electrode potential (-0.2 V) and referred to the spectra collected at a low potential (-0.6 V) at which adenine is not adsorbed. The spectra of adenine in solution are also shown in figure 3 for the sake of comparison. The spectra obtained with p-polarised light exhibit a bipolar feature at both pD conditions. The negative going band in the spectra collected with p-polarised light appear at the same wavenumbers as both the negative bands in the spectra obtained with s-polarised light and the bands in spectra of adenine in solution. Taking into account that the electric field of s-polarized light is cancelled at the reflection plane and, therefore, it only can interact with species in solution, the negative going bands must account for the loss of adenine in the thin layer of solution between the electrode and the CaF₂ window as a consequence of the adsorption on the electrode. The positive going p- bands, which account for the species adsorbed at the measurement potential, are slightly blue shifted as compared to the bands in solution as a consequence of the interaction with the electrode surface. The band observed at pD higher than pK_{a2} is c.a. 10 cm⁻¹ red-shifted from the corresponding band in neutral media. Moreover, the band obtained in basic media has a lower half-width value than the band in neutral or acid media. These observations suggest that each one of these two bands reflects the predominance of a different adsorbed species in the corresponding pD media.

- Fig. 3-

Some ATR-SEIRA spectra of adsorbed adenine on gold film electrodes obtained in neutral and basic media at a high potential are shown in figure 4. They are referred to the spectra obtained in the respective adenine-free supporting electrolytes at the same potential. The main signals at c.a. 1645 (neutral media) and 1636 cm^{-1} (basic media) exhibit the same differences as in the SNIFTIRS spectra: at $\text{pH} > \text{pK}_{a2}$ the band is red shifted and has a lower half-width than the corresponding band in neutral media, so confirming that it is caused by a different specie which must be the anionic adenine form (AdD_2^- in the D_2O solutions).

- Fig. 4 -

The absorption bands for the adsorbed uncharged adenine form (AdD_3) and for the adsorbed anionic form (AdD_2^-) show similar behaviours as a function of the electrode potential. They slightly shift towards higher wavenumbers as the potential increases, as consequence of the Stark effect (see Figure 4c). On the other hand, the amounts of adenine adsorbed from the two pH solutions follow the same potential dependence, as can be observed in Figure 4d from the normalized potential-dependent band heights for the signals corresponding to the two adenine forms.

3.4.- Evaluation of the pK_{a2} value for adsorbed adenine

In order to determine the pK_{a2} value for adenine at the electrode interface, an experiment was performed in which the pD of the solution in the spectro-electrochemical cell was varied in the range from 8 to 11, by adding successive spikes of a deuterated 0.1 M NaOH solution. In this way, possible artefacts caused by comparing experiments carried out with different gold film electrodes for each solution pD are avoided. In Figure 5a the gradual shift of the wavenumbers of the adsorbed adenine band from c.a. 1645 cm^{-1} at neutral pD to c.a. 1635 cm^{-1} at higher pD can be clearly seen. It can also be noticed that the half-width of the band decreases as the pD increases, from 12 – 15 cm^{-1} at neutral pD to 6-8 cm^{-1} at basic pD values, as the same time as the band height rises. All the spectra were obtained at the same electrode potential (0.2 V vs SCE)

It is reasonable to assume that each of the two bands obtained at the extreme pD values of the series in Figure 5a correspond to the absorption of a single adenine

form, the neutral form and the anionic unprotonated one. The evolution of the signal at intermediate pH values can be the result of the simultaneous absorption of both species on the electrode, connected by an acid-base equilibrium. According to this assumption, the band obtained at the intermediate pH values of the series must be the combination of the bands obtained at the extreme pH values. Figure 5b shows the deconvolution of the signal in the spectrum of the series corresponding to pH 9.8. It can be seen as the measured signal can be decomposed in two lorentzian bands at 1645 and 1637 cm^{-1} with the respective half-width values reported above.

- Fig. 5 -

It can be assumed that the integrated intensities of the signals at 1645 and 1627 cm^{-1} resulting from the deconvolution of the bands in the experimental spectra are proportional to the surface concentrations of the AdD_3 and AdD_2^- forms of adenine, respectively. The proportionality constants for the two forms include the integral of the transition dipole that can be different for both species. In order to compare the surface concentration of both species, the integrated values of each signal are normalised to their respective maximum values. The results of the thus normalized integral signals at 0.2 V vs SCE are plotted in Figure 5c as a function of pD. The plot shows the sigmoidal shapes expected for two conjugate species in an acid-base equilibrium. From the intercept of both plots a value of $\text{pK}_{a2}=9.8$ is obtained, the same as reported for adenine in solution. This value was found to be the same at different adsorption potentials investigated (from -0.2 V vs SCE to 0.2 V vs SCE). The agreement between the pK_{a2} values for adsorbed and dissolved adenine clearly indicates that the interaction sites of adenine molecule with the electrode surface are far from the site of the molecule involved in the protonation equilibrium, i.e. the nitrogen atom N_9 . This behaviour is contrary to what was observed for the pK_{a1} value of adsorbed adenine that decreases by more than 3 units of pH with respect to the value for adenine in solution, [4], suggesting that the molecular fragment involved in the first protonation equilibrium, i.e. the nitrogen atom N_1 , is close to the site of the molecule interacting with the electrode surface. As mentioned in the introduction section, in the model proposed for the adsorption of adenine the molecule interacts with the electrode by the amine nitrogen N_{10} and either the pyrimidine nitrogen N_1 or the imidazol nitrogen N_9 , which can explain the observed pH dependence.

3.5.-In situ FT-IRRAS of adsorbed adenine from neutral and basic H_2O solutions.

Some ATR-SEIRAS spectra collected for adsorbed adenine on gold film electrodes in neutral and basic H₂O solutions are given in Figure 6. The absorption bands observed in protonated media are wider and blue shifted as compared to the same bands in deuterated media, as a consequence of the presence of NH₂ scissoring, and probably because some OH bending signal from the solvent can be also present. On the other hand, differences observed in the signals obtained at each pH value are similar to those described for the external reflection spectra in D₂O. Namely, in basic media the band is slightly red shifted (1665 cm⁻¹) and has a lower half width (c.a. 20-25 cm⁻¹) than the corresponding band in neutral media (wavenumbers 1675 cm⁻¹ and half-width of 25-30 cm⁻¹)

It was previously observed [4] that adenine adsorption on gold from water acid solutions involve also the co-adsorption of the solvent, as inferred from the observed up-going character of the typical OH band in the region 3000-4000 cm⁻¹ (see the corresponding spectrum in Figure 6c).

The ATR-SEIRAS spectra for adsorbed adenine obtained from water solutions at pH higher than the pK_{a2} do not show the up-going O-H stretching band around 3600 cm⁻¹ assigned to non-hydrogen bonded OH form. The only up-going bands in the 3000-4000 cm⁻¹ region are at c.a. 3215 and 3370 cm⁻¹, and can be identified with the symmetric and anti-symmetric stretching of -NH₂. A down-going band appears at 3490 cm⁻¹ clearly indicating that water is displaced upon adenine adsorption in basic solutions. On the other hand, the co-adsorption of perchlorate was not detected in neutral solutions, contrary to what was observed for adenine adsorption from acid media [4]. The comparison of the spectra in the region 3000-4000 cm⁻¹ from acid, neutral and basic media given Figure 6c suggests that the hydronium ion is inducing the co-adsorption of water molecules.

4.- CONCLUSIONS

The in-situ FT-IRRAS experiments in basic D₂O solutions, at pH values significantly higher than the pK_{a2} of adenine, have revealed the adsorption of the anionic adenine form, AdD₂⁻, with somewhat different spectral characteristics than those of the uncharged adenine form, AdD₃. The intense ring stretching band in the spectral region 1600-1650 cm⁻¹ for the anionic form is red-shifted with respect to the corresponding signal for AdD₃ and is more intense and narrower.

The high resolution and sensitivity of the ATR-SEIRAS experiments allows the deconvolution of the mixed bands due to the ring stretching signals of the two co-

adsorbed adenine forms from solutions with pH values ranging from 8 to 11. From the relative normalized area of the two signals resulting from the deconvolution the pK_{a_2} value of adsorbed adenine was determined. The obtained value of 9.8 is the same as that reported for adenine in solution, independently of the adsorption potential. This result confirms the model previously proposed for adenine adsorption on gold electrodes, in which the atoms involved in the bonding with the metal are the nitrogen of the amino group, N_{10} , and the nitrogen N_7 (or N_1) of the ring, but not the nitrogen N_9 of the ring, which is involved in the second acid-base equilibrium of adenine.

In addition, the comparison of the spectra obtained in H_2O solutions at pH values ranging from 1 to 11 shows that co-adsorption of water molecules and of perchlorate anions of the supporting electrolyte take place only in acid media.

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Scheme 1.- Adenine structure with numeric labels and its acid base equilibria.

Figure 1.- Cyclic voltammograms of Au(111) electrodes in 1 mM adenine solutions at the indicated pH values. Scan rate = 50 mVs⁻¹. First voltammogram obtained with the freshly flame annealed electrodes (dashed lines) and stationary voltammograms (solid lines).

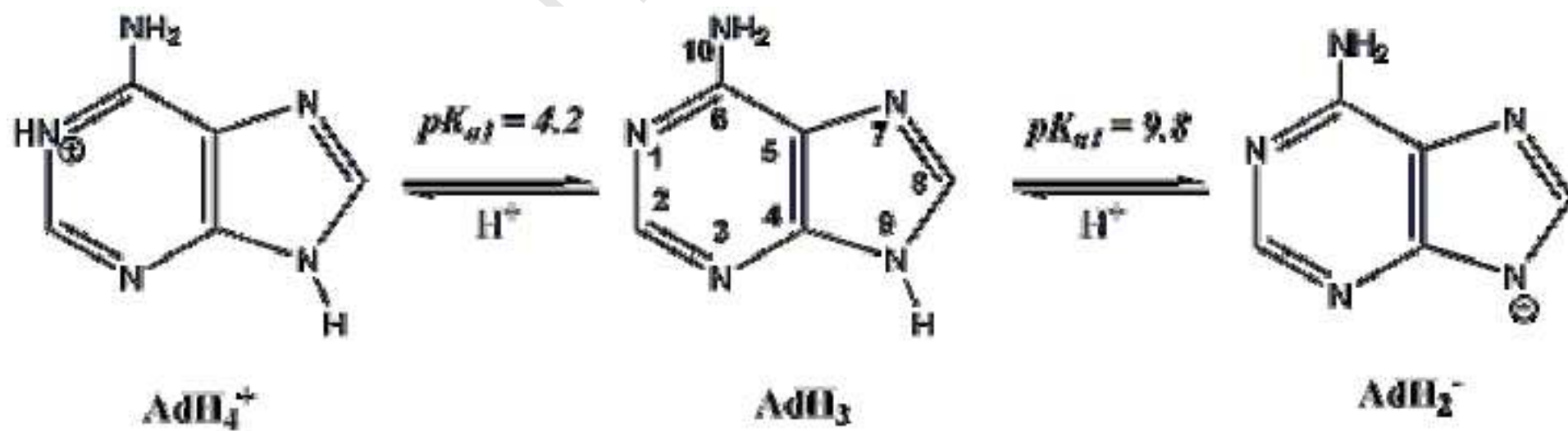
Figure 2.- Transmission spectra of 10 mM adenine in a) D₂O solutions and b) H₂O solutions at different pH/pD values: pH=7 (dash-dot line) and pH=11 (solid line)

Figure 3.- SNIFTIRS spectra for adenine on Au(111) at a potential (-0.2 V vs SCE) higher than the onset of adenine adsorption and referenced to a potential (-0.6 V vs SCE) at which adenine is not adsorbed, obtained with p (solid line) and s (dashed line) polarized lights from 10mM adenine solutions in D₂O at a) pD=7 and b) pD=11. The transmission spectra of deuterated adenine species in solution at the same pD values are included (dash-dot lines).

Figure 4.- ATR-SEIRAS spectra of adenine adsorbed on gold thin-film electrodes from 1 mM solutions in D₂O using R collected at a potential higher than the onset of adenine adsorption (0.2 V vs SCE) and R₀ collected at the same potential in the absence of adenine, and at a) pD 8 and b) pD 10.8; c) Potential dependence of the wavenumbers of the absorption band at c.a. 1640 cm⁻¹ obtained at pD=8 (circles) and pD=10.8 (triangles) and d) Normalized intensities of the bands used in c) as a function of the applied potential.

Figure 5.- a) ATR-SEIRAS spectra of adenine on gold thin-film electrodes from 1 mM solutions in D₂O at the indicated pH values. The spectra are obtained at 0.2 V vs SCE and referenced to the same potential in adenine-free supporting electrolyte; b) Absorption band at c.a. 1640 cm⁻¹ obtained from the spectrum at pD 9.8 in a) (solid black line) and calculated (dotted green line) from the combination of the signals at 1645 cm⁻¹ (blue dash-dot line) and 1636 cm⁻¹ (solid red line); c) Plots of the normalized integrated intensities of the absorption signals at 1645 cm⁻¹ (circles) and 1636 cm⁻¹ (triangles) resulting from the deconvolution of the absorption bands in a) and the best sigmoidal fitting to each plot (dash-dot and solid lines, respectively).

Figure 6.- ATR-SEIRAS spectra in the region of c.a. 1800 - 1500 cm⁻¹ of adenine adsorbed on gold thin-film electrodes from 1 mM solutions in H₂O at different pH values. Reflectance spectra obtained at a high potential (0.2 V vs SCE for spectra in neutral and basic media and 0.33 V for the spectrum in acid media) and referenced to the reflectance collected at the same potential in the absence of adenine: a) at pH=8; b) at pH=10.8. c) Spectral region of c.a. 4000-3000 cm⁻¹, obtained in the same way that in a) and b) at the three indicated pH values.



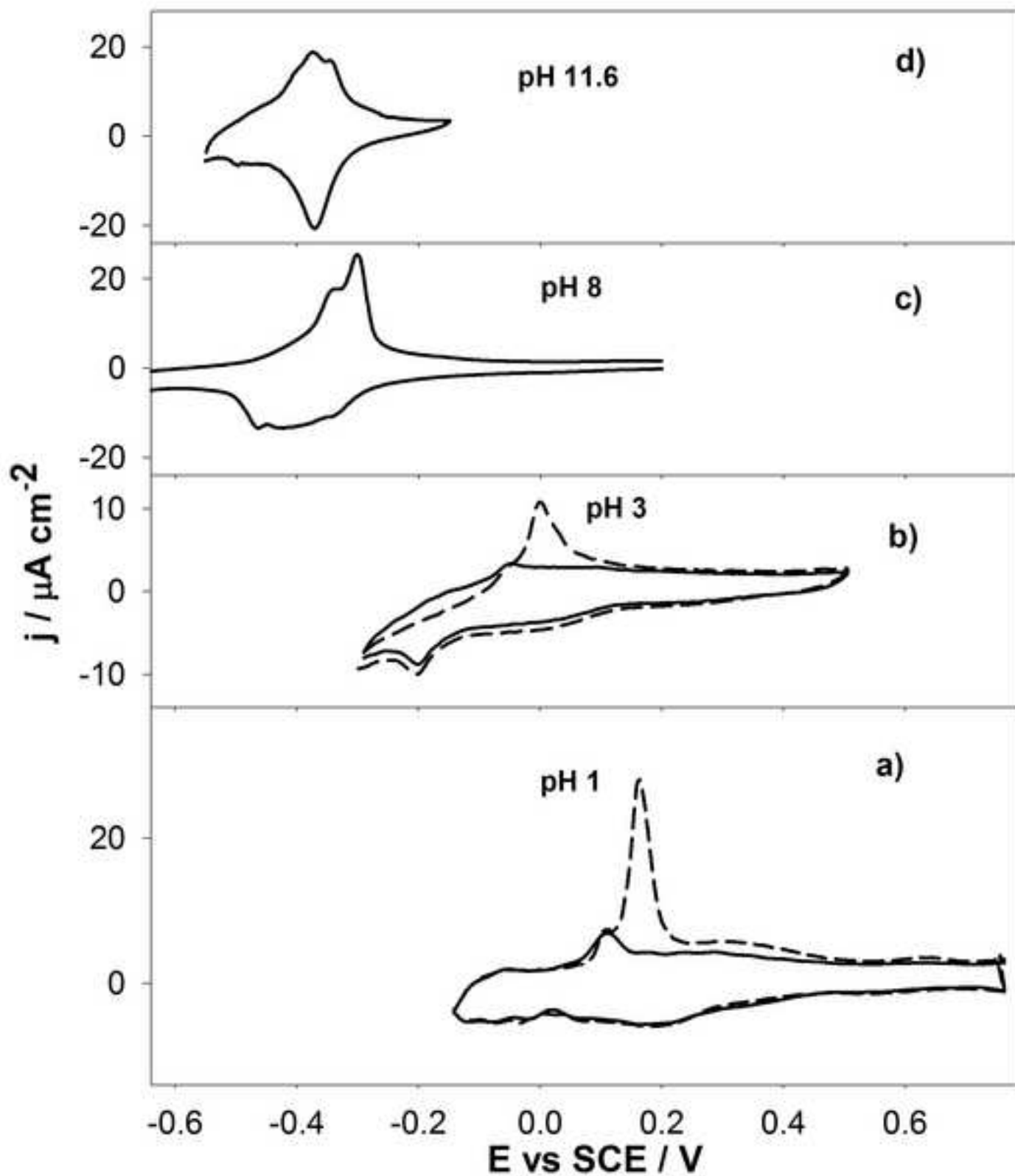
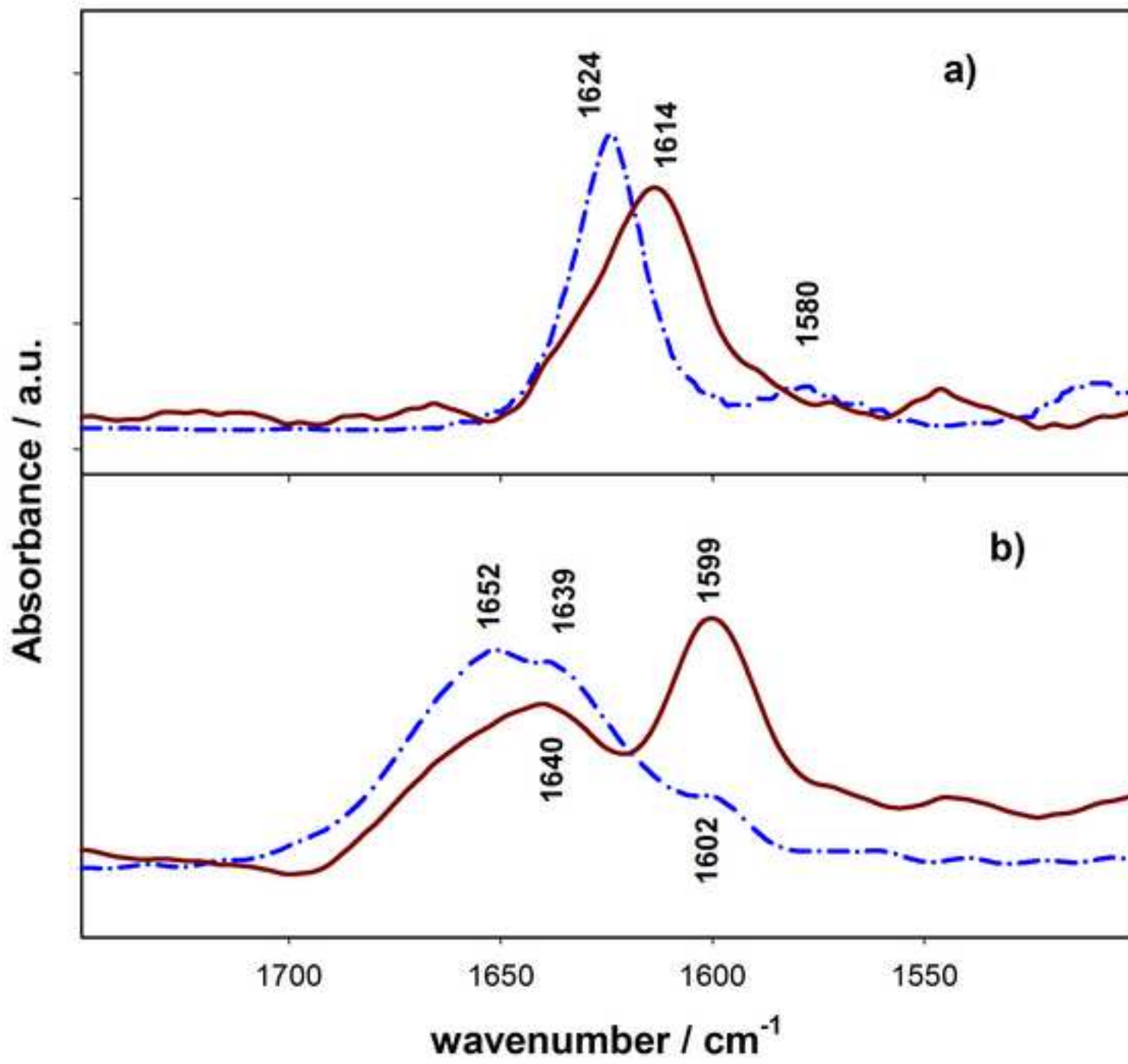
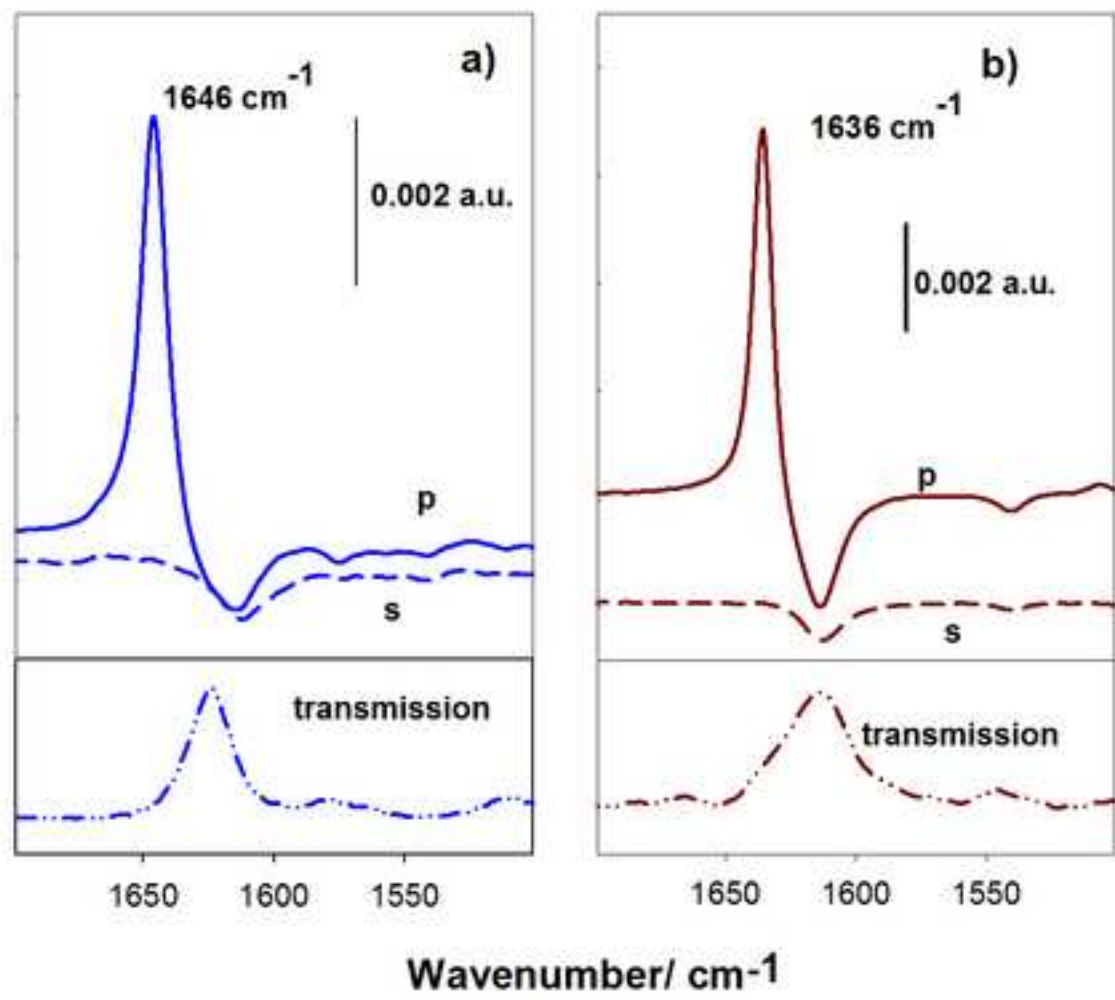
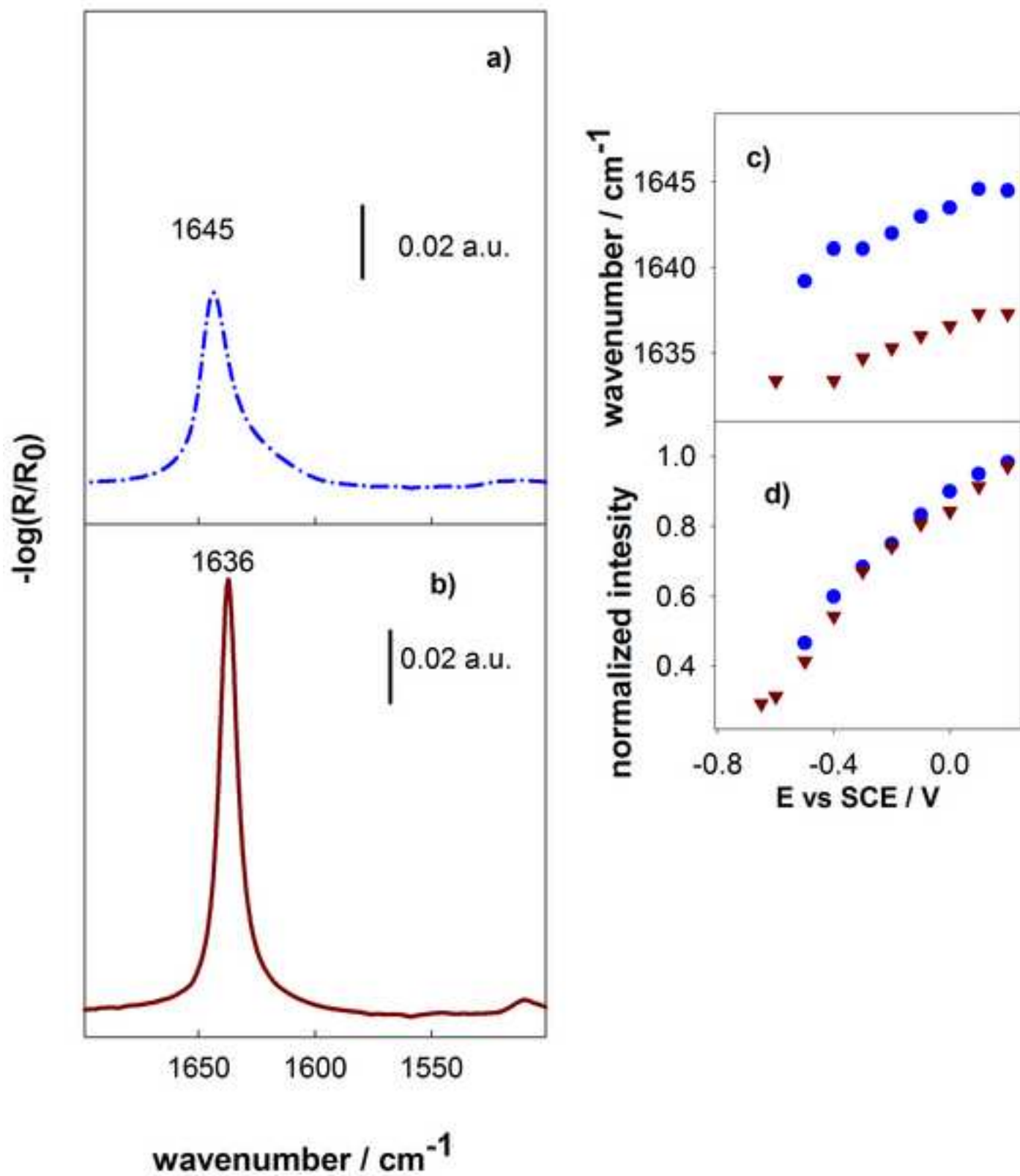


Figure 2



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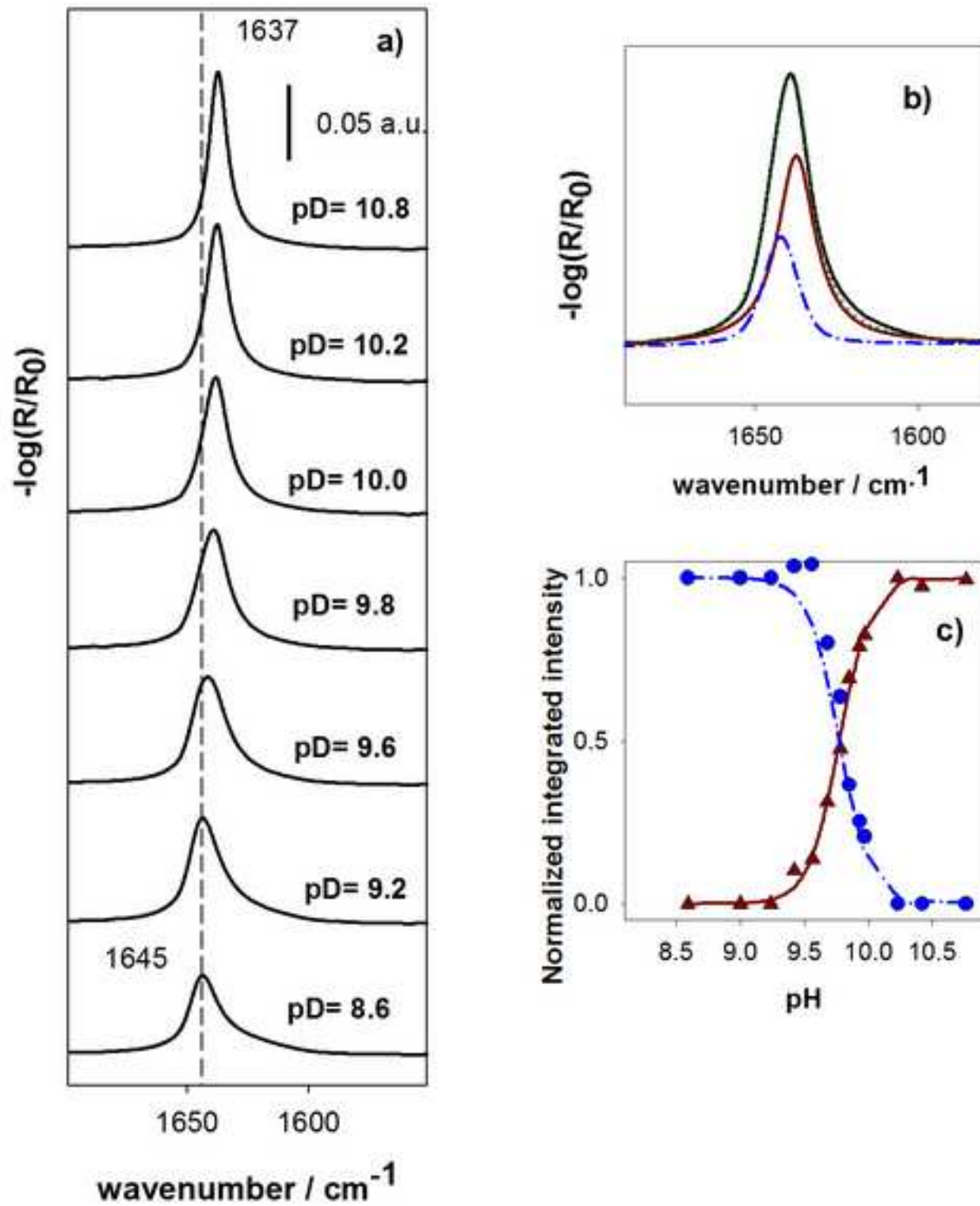


Figure 6

