

Raman study on the structure of adlayers formed on silver from mixtures of 2-aminoethanethiol and 3-mercaptopropionic acid

Beata Wrzosek, Jolanta Bukowska and Andrzej Kudelski*

Warsaw University, Department of Chemistry, Pasteur 1, 02-093 Warsaw, Poland

Received 30 March 2005; Accepted 28 June 2005

Silver surfaces were covered with adlayers formed from mixtures of 2-aminoethanethiol (cysteine, CYS) and 3-mercaptopropionic acid (MPA). The composition and structure of adlayers thus formed were investigated by surface-enhanced Raman scattering (SERS). SERS measurements revealed that the layer formed from the 1:1 mixture of 10-mM aqueous solutions of CYS and MPA is composed of co-adsorbed CYS and MPA molecules. From the SERS spectrum of such an adlayer, one can also deduce that practically all MPA molecules are dissociated. pH-controlled experiments showed that MPA is also dissociated when mixed MPA + CYS adlayer is soaked in the solution buffered to pH = 5.0, whereas in the case of one-component adlayer, almost all MPA molecules are protonated at the same pH value. It means that the presence of CYS molecules induces dissociation of neighbouring MPA molecules and that co-adsorbed MPA and CYS probably form mixed salt-like structures under these conditions. At lower pH values (3.2), the layer is mainly composed of non-dissociated MPA molecules, while at slightly alkaline solution (pH = 9) strong preference of CYS molecules at the surface adlayer is observed. This phenomenon may be ascribed to strong stabilization of the adlayer structure by the network of hydrogen bonds between COOH (at lower pH) or NH₂ (at higher pH) groups of adjacent molecules. Formation of mixed adlayer is possible in the limited range of pH values that enable protonation of NH₂ groups with simultaneous deprotonation of the COOH groups. Our results also demonstrate that the mixed salt-like MPA + CYS adlayers could be formed from the MPA + CYS solutions only if the concentrations of both compounds are of the same order of magnitude. This suggests no strong thermodynamic preference for such adlayers. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: SERS; self-assembly; mixed monolayers; omega-terminated alkanethiols

INTRODUCTION

Alkanethiols or dialkyl disulphides are among the most successful chemicals employed as attachment layers to metals, because they react with many metals (including gold, silver, platinum) forming very stable metal–sulphur bonds.^{1–4} The metal surfaces modified with adlayers formed from these organosulphur compounds have attracted considerable attention over the past years because of very large technological applications of such systems. The following representative applications of metals modified with organic adlayers may serve to illustrate their wide technological

importance: construction of chemical^{5,6} and biochemical^{7,8} sensors; providing microenvironments on metal surfaces similar to biological membranes that allows adsorption of proteins without denaturation,^{7,9} immobilization of DNA^{10,11} or adhesion of living cells to the metal surface;^{7,12} preparation of surfaces that resist the adsorption of proteins and cell attachment;^{12,13} preparation of surfaces for controlled growth of crystals;¹⁴ micro contact printing of metals;^{15,16} protection of metals against corrosion;^{17,18} anti-adherent coatings of metal surfaces.¹⁹

To prepare the metal substrates (as electrodes) for some applications, the surface of the metal is modified with the organic adlayer formed from substituted alkanethiols (typically ω -substituted: HS-(CH₂)_n-X). In adlayers formed from such compounds, the attached X group significantly influences the surface properties of the adlayer. The properties of the organic adlayers that are used for the modification of

*Correspondence to: Andrzej Kudelski, The University of Warsaw, Department of Chemistry, Pasteur 1, 02-093 Warsaw, Poland.
E-mail: akudel@chem.uw.edu.pl

Contract/grant sponsor: Ministry of Scientific Research and Information Technology; Contract/grant number: PBZ 18-KBN-098/T09/2003.

the metal substrates can be, in some cases, more precisely engineered for various purposes by using a mixture of two or more thiols in the construction of the adlayer. Mixed adlayers can be formed from alkanethiols terminated with different groups^{20–22} and/or from compounds with different lengths of the alkane chains.^{21,22}

Adlayers containing, in the outermost part, both amino and carboxylic groups are sometimes used in practice, e.g. for immobilizing biologically important molecules. For example, on such adlayers, Ataka and Heberle²³ immobilized cytochrome *c*, and Disley *et al.*²⁴ immobilized immunoglobulin G.

The objective of the investigations described in this paper was to determine the composition and structure of adlayers formed on silver surfaces from various mixtures of cysteamine (2-aminoethanethiol, HS-(CH₂)₂-NH₂, abbreviated as CYS) and 3-mercaptopropionic acid (HS-(CH₂)₂-COOH, abbreviated as MPA). The influence of pH of the solution on the structure of formed adlayers is also analysed. We hope that such studies might be useful in choosing the proper parameters for formation of mixed CYS + MPA adlayers.

In this paper, the structure of thiolate adlayers formed on silver has been determined with surface-enhanced Raman spectroscopy (SERS). The Raman cross section of molecules adsorbed on roughened silver surfaces increases by many orders of magnitude making SERS one of the most sensitive tools for studying the orientation and conformation of adsorbed molecules.^{25–30}

EXPERIMENTAL

All chemicals were purchased from commercial suppliers (Sigma–Aldrich, Merck, POCH) and were used as received. CYS (from Sigma–Aldrich), and 3-mercaptopropionic acid (from Merck) were of reagent grade, whereas all inorganic chemicals were of analytical reagent grade. Distilled water was further purified with a Millipore Mill-Q water system (final resistivity ~18 MΩ cm).

Before Raman measurements, the silver substrates for the organic film deposition were electrochemically roughened to obtain a sufficiently enhanced intensity of the SERS bands. Electrochemical roughening was carried out in a conventional three-electrode cell with a large platinum sheet as the counter-electrode and 0.1 M KCl AgCl|Ag electrode as the reference (all potentials are quoted versus this electrode). The silver electrodes were roughened by three successive positive–negative cycles in a 0.1-M KCl aqueous solution from –0.3 to 0.3 to –0.3 V at a sweep rate of 5 mV s^{–1}. The cycling was finished at –0.3 V, then the applied potential was changed to –0.4 V and the silver electrode was kept for 5 min at this potential; after that, the working electrode was removed at an open circuit potential and very carefully rinsed with water.

Raman spectra were recorded with an ISA T64000 (Jobin–Yvon) Raman spectrometer equipped with a Kaiser

holographic notch filter (SuperNotch-Plus HSPF-647.1-1.0), 1800 (or 600) grooves/mm holographic grating, an Olympus BX40 microscope with a 50 × long-distance objective (LMPLFL50×/0.50), and 1024 × 256 pixels, nitrogen-cooled CCD detector. A Laser-Tech model LJ-800 mixed argon/krypton laser provided excitation radiation of 647.1 nm.

RESULTS AND DISCUSSION

Figure 1 shows SERS spectra of adlayers formed on silver from a 10-mM aqueous solution of MPA (natural pH = 3.5) and from a 10-mM aqueous solution of CYS (natural pH = 10.7) in the wavenumber range 550–1200 cm^{–1}. The adlayers were formed by immersion of the silver substrates in the MPA or CYS solution for 2 h. SERS measurements were carried out for metal substrates still immersed in the solution from which the organic adlayer was formed.

As can be seen from Fig. 1, in this wavenumber range the SERS spectrum of the MPA adlayer is dominated by the band at 906 cm^{–1}. According to Moskovits and Suh,³¹ this band should be assigned to the C–COOH stretching

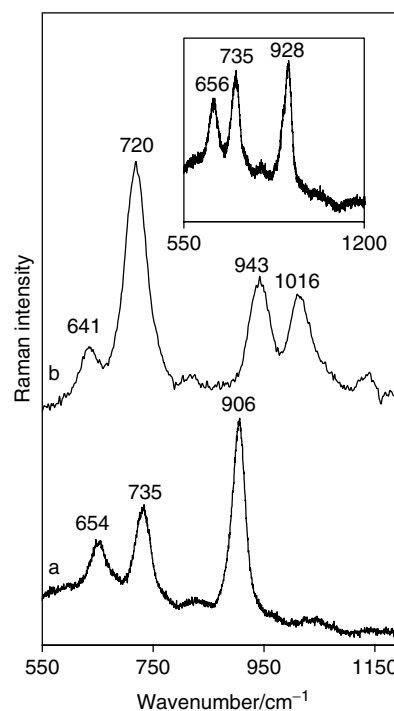


Figure 1. SERS spectra of adlayers formed on silver from: (a) 10-mM aqueous solution of MPA (pH = 3.5) and (b) 10-mM aqueous solution of CYS (pH = 10.7). The adlayers were formed by immersion of the silver substrates in a solution of MPA or CYS for 2 h. SERS measurements were carried out for metal substrates still immersed in the solution from which the organic adlayer was formed. Spectra are scaled and vertically shifted for clarity. Inset: SERS spectrum of the MPA adlayer formed from 10-mM solution of MPA buffered to pH = 8.2.

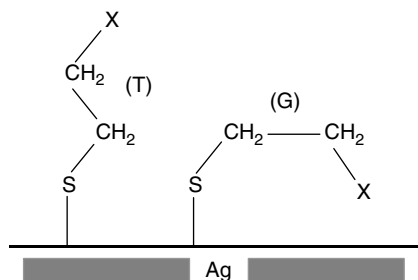


Figure 2. Possible structures of S–C–C–X chain: (T) trans conformation, (G) gauche conformation. X = COOH or NH₂ for MPA and CYS respectively.

vibration. The increase of pH of the solution in which the metal substrate covered with the MPA adlayer is immersed, leads to disappearance of this band and to the appearance of the band due to the C–COO[−] stretching vibration at 928 cm^{−1} (inset in Fig. 1). As follows from our previous results, comparable intensities of the 906 and 928 cm^{−1} bands are observed³² at pH values in the range 5.8–6.2 (on the basis of the relative intensities of the 906/928 cm^{−1} bands one can roughly estimate the degree of dissociation of chemisorbed MPA – for details see Ref. 32). Two bands at about 700 cm^{−1}, which are also clearly seen in the SERS spectra of MPA, are due to stretching vibrations of the C–S bonds. The band at the lower wavenumber (654 cm^{−1}) is characteristic for a gauche conformer of the S–C–C–C chain, whereas the band at higher wavenumber (735 cm^{−1}) is typical of a trans conformer.^{32–42} Structures of the S–C–C–C chain in trans and gauche conformation are presented in Fig. 2. The relative intensity of the $\nu(\text{C–S})_G$ and $\nu(\text{C–S})_T$ bands does not change significantly upon the dissociation of carboxylic groups of MPA (Fig. 1).

In the SERS spectra of CYS adlayer the $\nu(\text{C–S})$ bands due to gauche and trans conformers of the S–C–C–N chain appear at 641 and 720 cm^{−1}, respectively (Fig. 1). The bands at 943 and 1016 cm^{−1}, which can be also clearly seen in the SERS spectrum of CYS adlayer, are due to the C–C stretching vibration coupled to the C–N stretching vibration.³⁷

Figure 3 shows the SERS spectra of the organic adlayers formed on silver from the 1:1 mixture of 10-mM aqueous solutions of CYS and MPA buffered to pH = 5 and to pH = 7.2 (see inset). We decided to work only with buffered solutions because small excess of one compound (CYS or MPA) would significantly change the pH of the almost equimolar mixture of CYS and MPA (pH of the actual equimolar mixture of CYS and MPA should be very close to 7). As may be seen in Fig. 3, in these SERS spectra one can identify five bands: at 656, 730, 925, 945 and 1018 cm^{−1}; however, the bands at 925, 945 cm^{−1} are not well resolved in some spectra and one band centred at about 936 cm^{−1} alone could be observed in these cases. It is worth stressing that the half-widths of both the 936 cm^{−1} band and the

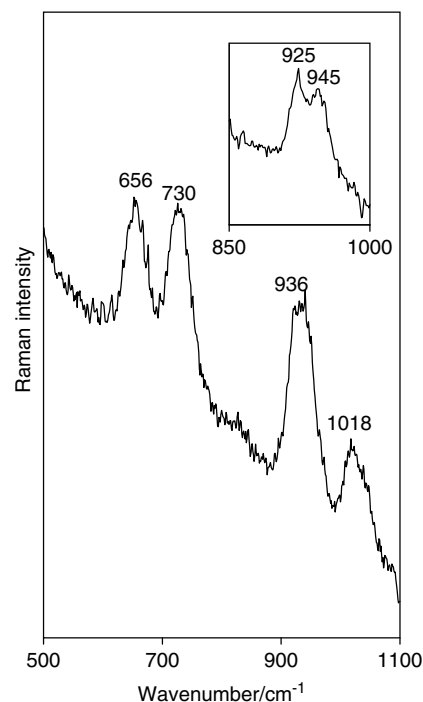


Figure 3. SERS spectrum of the organic adlayer formed on silver from the 1:1 mixture of 10-mM aqueous solutions of CYS and MPA (just-obtained mixture was buffered to pH = 5). The adlayer was formed by the immersion of silver substrate in the MPA + CYS solution for 2 h. SERS measurement was carried out for the metal substrate still immersed in the solution from which the organic adlayer was formed. Inset: SERS spectrum of the CYS + MPA adlayer on silver measured for the sample immersed in the solution buffered to 7.2 (the pH was controlled using the phosphate buffer).

doublet shown in the inset of Fig. 3 are comparable (about 50 cm^{−1}) suggesting that the 936-cm^{−1} feature is composed of overlapped 925 cm^{−1} (for MPA, $\Delta\nu_{1/2} = 30$ cm^{−1}) and 945 cm^{−1} (for CYS, $\Delta\nu_{1/2} = 40$ cm^{−1}) components of almost equal intensities. Thus, in the presented SERS spectra of the adlayers formed from the equimolar mixture of CYS and MPA, in addition to the non-characteristic $\nu(\text{C–S})_G$ and $\nu(\text{C–S})_T$ bands (at 656 and 730 cm^{−1}, respectively) one can also distinguish bands, which appear, to a large extent selectively, in the SERS spectrum of the CYS adlayer (at 945 and 1018 cm^{−1}) and in the spectrum of MPA molecules with dissociated carboxylic groups (the component at 925 cm^{−1}).

Spectra of molecules adsorbed in various conformations are significantly different. In the reference spectrum of CYS adlayer (Fig. 1), the ratio of intensities of $\nu(\text{C–S})_T$ and $\nu(\text{C–S})_G$ bands is about 5.5, whereas, for the adlayer formed from the 1:1 mixture of 10-mM aqueous solutions of CYS and MPA, the ratio is about 1.3. Therefore, we decided to check whether (and if so, how) the spectrum of CYS molecules in the C–C + C–N stretching region (900–1050 cm^{−1}) changes when the relative surface concentration of the gauche

conformer increases. We also wanted to check whether the spectrum of CYS molecules changes upon protonation.

As has been previously shown, desorption of the part of adsorbed CYS molecules leads to a significant increase of the ratio of surface concentrations of gauche to trans conformers^{32,37} (CYS molecule in the gauche conformation takes more 'surface space' than in the trans conformation; see Fig. 2). Therefore, we measured the temporal evolution of the SERS spectrum of the CYS adlayer on silver substrate immersed in the pure water. We found that increase in the relative surface concentration of the gauche conformer during the partial desorption of CYS molecules results in a significant decrease of the relative intensity of the band at about 945 cm^{-1} ; however, no new components at about 905 or 930 cm^{-1} (which could be later misinterpreted as a contribution from MPA molecules) appear (Fig. 4b). The increase of the ratio of surface concentrations of gauche to trans conformers could be also achieved by addition of phosphates (used in other experiments to control pH) to the surrounding solution (inset in Fig. 4). SERS spectrum of the CYS adlayer immersed in the solution containing phosphates is similar to that of the adlayer with the relative surface concentration of molecules in the trans conformation decreased by the partial desorption of CYS molecules (compare spectrum Fig. 4b with the spectrum in the inset in Fig. 4). One can also expect changes in the spectrum of CYS adlayer after protonation of amino groups. We found, however, that after the protonation of CYS molecules (Fig. 4c), only a very weak, new feature at 886 cm^{-1} is seen in this region of the spectrum, far away from the 925 cm^{-1} band. Assignment of the bands at 886 and 840 cm^{-1} (which appears in Fig. 4b) is unclear. For chelate complex of CYS with Hg(II), the bands observed in the wavenumber range of $800\text{--}900\text{ cm}^{-1}$ were ascribed⁴³ to mixed vibrations involving $\delta(\text{HCC/HCS}) + \nu(\text{CN})$ – band at 822 cm^{-1} and $\delta(\text{oopNH}_2) + \nu(\text{CC})$ – band at 888 cm^{-1} . Similar pH and desorption experiments have been also carried out for the MPA adlayers. We found that after partial desorption of MPA molecules and for MPA adlayers immersed in various buffer solutions, no new component appears that could correspond to the 945 and 1018 cm^{-1} features observed in the spectrum of mixed adlayer. Thus, it can be concluded that the band at 925 cm^{-1} in the spectrum of the adlayer formed from the mixture of CYS and MPA must be due to dissociated MPA molecules, whereas the bands at 945 and 1018 cm^{-1} are undoubtedly due to the CYS molecules. In this case chemisorbed MPA molecules are practically completely dissociated because the band at 906 cm^{-1} , which is characteristic for non-dissociated MPA, is not observed at all.

Previous SERS studies of the MPA adlayers showed³² that the intensity of the band at 906 cm^{-1} becomes equal to the intensity of the band at 928 cm^{-1} if the adlayer is immersed in the solutions with $\text{pH} \approx 5.8\text{--}6.2$. For adlayers formed from the 1 : 1 mixture of MPA and CYS buffered to $\text{pH} = 5.0$, we

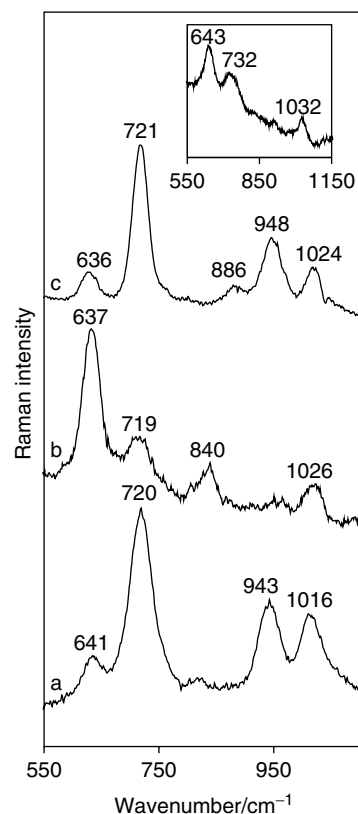


Figure 4. SERS spectra of CYS adlayers on silver. (a) Freshly formed adlayer (for details see caption to Fig. 1), (b) the adlayer with surface concentration of CYS decreased by 5 h of soaking in pure water (c) CYS adlayer immersed in 10-mM CYS solution buffered to $\text{pH} = 3.2$. The spectra have been scaled and vertically shifted to enhance clarity. Inset: SERS spectrum of the CYS adlayer formed from 10-mM solution of CYS buffered to $\text{pH} = 10$ using phosphate buffer (total concentration of Na_3PO_4 and Na_2HPO_4 in the solution was equal to 0.1 M).

found, however, that the component at about 905 cm^{-1} (due to the C–COOH stretching vibration) does not observably contribute to the broad band centred around 936 cm^{-1} (Fig. 3), whereas the component at 925 cm^{-1} , $\nu(\text{C–COO}^-)$, undoubtedly contributes. This observation suggests that in the mixed CYS + MPA adlayer MPA does not form large domains (the properties of domains of MPA molecules, which do not contain CYS molecules, should be very similar to the properties of MPA in the 'pure' MPA adlayer).

In the SERS spectrum of an adlayer formed from the equimolar mixture of CYS and MPA buffered to 3.2 (Fig. 5e), the relative intensity of the bands characteristic for CYS molecules (945 and 1020 cm^{-1}) dramatically drops down. The recorded spectrum is very similar to that of the adlayer formed from non-dissociated MPA molecules and one can assume that in such conditions the adlayer is almost selectively formed from non-dissociated MPA with small admixture of CYS molecules. This is probably due

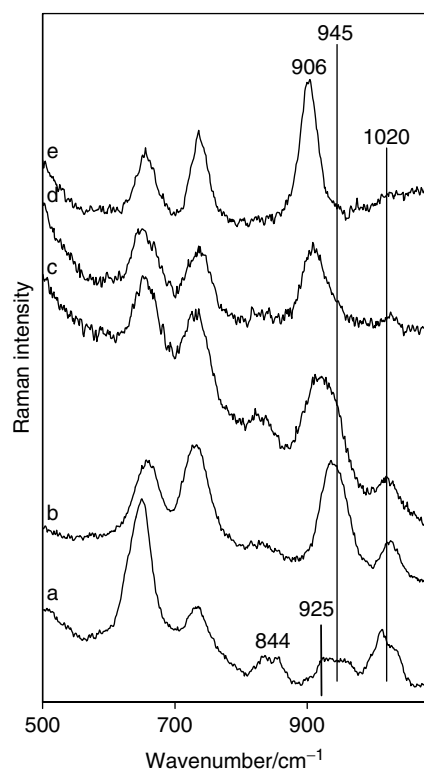


Figure 5. SERS spectra of adlayers formed from the 1 : 1 mixture of 10-mM aqueous solutions of CYS and MPA buffered to: (a) pH = 9.0; (b) 7.2; (c) 4.5; (d) 3.9; (e) 3.2. The pH was controlled with various phosphate buffers (as previously shown in Ref. 45, in these conditions contribution from phosphate anions to the measured SERS spectrum is practically negligible). The spectra have been scaled and vertically shifted to enhance clarity.

to strong stabilization of such an adlayer by the network of hydrogen bonds between non-dissociated carboxylic groups. On the other hand, the SERS spectrum of an adlayer formed from an equimolar mixture of CYS and MPA buffered to pH 9 (Fig. 5a) is generally similar to that of the adlayer formed from CYS molecules. However, the SERS spectrum of the adlayer formed from the CYS + MPA mixture reminds one of that of the CYS adlayer shown in Fig. 4b, thus indicating considerably higher amount of the gauche conformers than that observed in the SERS spectrum of CYS adlayer formed from 10-mM CYS solution. These differences are perhaps caused by the presence of anions from the buffer solution in experiment with mixed monolayer since, as previously shown,⁴⁴ different ionic strength may influence the conformation of adsorbed CYS molecules. As can be seen in Fig. 5a, the 925 cm⁻¹ band, characteristic of dissociated MPA is very weak, pointing to a rather small number of MPA molecules in the mixed adlayer (compare inset in Fig. 1). Thus, one may assume that in alkaline solutions the adlayer is almost selectively formed from CYS with a small admixture of MPA molecules. Thus, it seems

that in moderately alkaline medium (pH = 9), when CYS molecules have neutral amine groups but MPA molecules are negatively charged, formation of hydrogen bonds between NH₂ groups leads to strong preference of CYS adsorption. This situation is similar to that found for slightly acidic (pH = 3.2) solution in which an almost one-component adlayer consisting protonated MPA molecules are formed. Formation of mixed adlayer is facilitated by electrostatic interaction of oppositely charged CYS and MPA molecules. However, as evidenced by our experiments, this is only possible in rather narrow pH range (from about 4 to 8).

It is important to note that from the analysis of the SERS spectra, we were not able to determine if CYS molecules are protonated in the mixed adlayer or not, because of general similarity of the CYS spectra for pH = 10.7 (Fig. 4a,) and for pH = 3.2 (Fig. 4c). This should be possible from the analysis of the SERS spectrum in the N–H stretching region, but we were unable to record any reliable SERS bands due to the N–H stretching vibrations. However, at pH = 5 co-adsorption of neutral CYS molecules with dissociated MPA seems to be unlikely. Thus, we can safely assume that mixed MPA-CYS adlayers have salt-like structure.

We also analysed the dependence of the composition of formed adlayer on the ratio of concentrations of MPA and CYS in the modifying solution. As has been shown earlier, the composition of the adlayer depends on pH of the solution from which the adlayer is deposited. Therefore, all experiments have been carried out using buffered solutions. We found that in the SERS spectrum of the adlayer formed from the 5 : 1 mixture of CYS and MPA buffered to 5.0 (Fig. 6b) both 945 and 1020 cm⁻¹ bands characteristic of cysteamine are observed alongside, with the 925 cm⁻¹ component clearly visible on the low-wavenumber side of the broad 945 cm⁻¹ band ($\Delta\nu_{1/2} \approx 55$ cm⁻¹). Similarly, for reversed CYS to MPA concentration ratio (1 : 5), bands characteristic of dissociated MPA molecules (~ 925 cm⁻¹) and that characteristic for CYS (1020 cm⁻¹) are seen in the spectrum (See Fig. 6d). The band centred at 925 cm⁻¹ is asymmetric on the high-wavenumber side and its half-width (~ 60 cm⁻¹) is considerably greater than that observed for $\nu(\text{C}-\text{COO}^-)$ band at 928 cm⁻¹ for pure MPA monolayer (~ 30 cm⁻¹). These observations suggest that for 1 : 5 or 5 : 1 (CYS : MPA) mixtures the adlayers formed at the Ag surface contain both molecules. However, when higher excess of one component (CYS or MPA) was present in the solution used for adlayer formation at the same pH value, the spectral pattern considerably changed. As seen in the SERS spectrum of the adlayer formed from the 10 : 1 mixture of MPA and CYS, the bands at 945 and 1020 cm⁻¹, which are characteristic for CYS, do not appear, whereas the band at 928 cm⁻¹ (typical for dissociated MPA) is clearly visible (Fig. 6e). On the other hand, in the SERS spectrum of the adlayer formed from the 1 : 10 mixture of MPA and CYS (also buffered to 5.0) one can only see bands characteristic for CYS, whereas, the component characteristic of dissociated MPA (at 928 cm⁻¹) does not appear (Fig. 6a). This points out

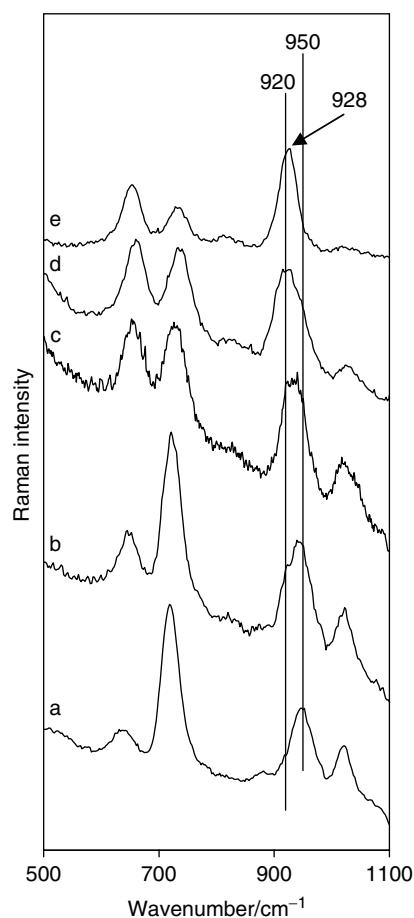


Figure 6. SERS spectra of adlayers formed from various mixtures of 10-mM aqueous solutions of CYS and MPA. All solutions were buffered with phosphate buffer to pH = 5. The ratio of CYS and MPA in the mixture: (a) 10 : 1, (b) 5 : 1, (c) 1 : 1, (d) 1 : 5, (e) 1 : 10. The spectra have been scaled and vertically shifted to enhance clarity.

that there is no strong thermodynamic preference for the mixed salt-like MPA-CYS adlayer.

CONCLUSIONS

The composition and structure of organic adlayers formed on silver surfaces from mixtures of CYS and MPA were investigated by SERS. We found that in recorded SERS spectra one can clearly distinguish bands characteristic for both MPA and CYS molecules. Moreover, owing to the fact that the SERS spectra for neutral and dissociated MPA molecules are considerably different, one can roughly estimate the degree of dissociation of chemisorbed MPA in the adlayer thus formed. Raman measurements of the adlayer formed from the 1:1 mixture of 10-mM aqueous solutions of CYS and MPA revealed that MPA molecules do not form domains at the surface and that the outermost part of the adlayer has probably mixed, salt-like structure:

$(-\text{NH}_3^+) \cdot (-\text{COO}^-)$. CYS does not practically co-adsorb with MPA from the solution containing 1:1 mixture of CYS and MPA buffered to pH = 3.2. In this case, the adlayer is almost selectively formed from non-dissociated MPA. On the other hand, an adlayer formed from the equimolar mixture of CYS and MPA buffered to 9 is almost selectively formed from CYS with a small admixture of MPA molecules. Most probably in both cases there is a strong stabilization of the adlayer structure by the network of hydrogen bonds between neutral amino or non-dissociated carboxylic groups. Also, adlayers formed from the 10:1 and 1:10 mixtures of MPA and CYS are constructed mainly from MPA or CYS molecules respectively. It means that mixed salt-like MPA + CYS adlayer could be only formed from MPA + CYS solutions having pH and the ratio of concentrations of MPA and CYS in a relatively narrow range. These results demonstrate that the formation of the salt-like mixed MPA-CYS adlayer from the mixture of MPA and CYS, is not strongly preferred thermodynamically.

Acknowledgments

The authors express their gratitude to Dr Agnieszka Michota-Kamińska for very valuable technical assistance. This work was supported by the Ministry of Scientific Research and Information Technology in 2004–2007, Project No. PBZ 18-KBN-098/T09/2003.

REFERENCES

1. Ulman A. *Chem. Rev.* 1996; **96**: 1533.
2. Fendler JH. *Chem. Mater.* 2001; **13**: 3196.
3. Whitesides GM, Laibinis PE. *Langmuir* 1990; **6**: 87.
4. Schreiber F. *Prog. Surf. Sci.* 2000; **65**: 151.
5. Berger R, Delamarche E, Lang HP, Gerber C, Gimzewski JK, Meyer E, Güntherodt HJ. *Science* 1997; **276**: 2021.
6. Dermody DL, Lee Y, Kim T, Crooks RM. *Langmuir* 1999; **15**: 8435.
7. Ostuni E, Yan L, Whitesides GM. *Colloids Surf. B* 1999; **15**: 3.
8. Cooper MA. *Nat. Rev. Drug Discov.* 2002; **1**: 515.
9. Houseman BT, Huh JH, Kron SJ, Mrksich M. *Nat. Biotechnol.* 2002; **20**: 270.
10. Sortino S, Petralia S, Condorelli GG, Conoci S, Condorelli G. *Langmuir* 2003; **19**: 536.
11. Higashi N, Takahashi M, Niwa M. *Langmuir* 1999; **15**: 111.
12. Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. *Science* 1997; **276**: 1425.
13. Ostuni E, Chapman RG, Liang MN, Meluleni G, Pier G, Ingber DE, Whitesides GM. *Langmuir* 2001; **17**: 6336.
14. Aizenberg J, Black AJ, Whitesides GH. *J. Am. Chem. Soc.* 1999; **121**: 4500.
15. Love JC, Wolfe DB, Chabinyc ML, Paul KE, Whitesides GM. *J. Am. Chem. Soc.* 2002; **124**: 1576.
16. Abbott NL, Kumar A, Whitesides GM. *Chem. Mater.* 1994; **6**: 596.
17. Laibinis PE, Whitesides GM. *J. Am. Chem. Soc.* 1992; **114**: 9022.
18. Azzaroni O, Cipollone M, Vela ME, Salvarezza RC. *Langmuir* 2001; **17**: 1483.
19. Azzaroni O, Schilardi PL, Salvarezza RC. *Nano Lett.* 2001; **1**: 291.
20. Bain CD, Evall J, Whitesides GM. *J. Am. Chem. Soc.* 1989; **111**: 7155.
21. Bain CD, Whitesides GM. *J. Am. Chem. Soc.* 1989; **111**: 7164.
22. Laibinis PE, Whitesides GM. *Langmuir* 1990; **6**: 87.
23. Ataka K, Heberle J. *J. Am. Chem. Soc.* 2004; **126**: 9445.
24. Disley DM, Cullen DC, You HX, Lowe CR. *Biosens. Bioelectron.* 1998; **13**: 1213.

25. Otto A, Mrozek I, Grabhorn H, Akemann W. *J. Phys. Condens. Matter* 1992; **4**: 1143.
26. Tian ZQ, Ren B, Wu DY. *J. Phys. Chem. B* 2002; **106**: 9463.
27. Otto A. *J. Raman Spectrosc.* 1991; **22**: 743.
28. Cotton TM, Kim JH, Chumanov GD. *J. Raman Spectrosc.* 1991; **22**: 729.
29. Kneipp K, Kneipp H, Itzkan I, Dasari RR, Feld MS. *J. Phys. Condens. Matter* 2002; **14**: R597.
30. Campion A, Kambhampati P. *Chem. Soc. Rev.* 1998; **27**: 241.
31. Moskovits M, Suh JS. *J. Am. Chem. Soc.* 1985; **107**: 6826.
32. Kudelski A. *J. Raman Spectrosc.* 2003; **34**: 853.
33. Dick LA, Haes A, Van Duyne RP. *J. Phys. Chem. B* 2000; **104**: 11 752.
34. Castro JL, Lopez-Ramirez MR, Arenas JF, Otero JC. *J. Raman Spectrosc.* 2004; **35**: 997.
35. Bryant MA, Pemberton JE. *J. Am. Chem. Soc.* 1991; **113**: 8284.
36. Smejkal P, Vlckova B, Prochazka M, Mojzes P, Plfeger J. *Vib. Spectrosc.* 1999; **19**: 243.
37. Kudelski A, Hill W. *Langmuir* 1999; **15**: 3162.
38. Kudelski A. *Langmuir* 2003; **19**: 3805.
39. Joo TH, Kim K, Kim MS. *J. Phys. Chem.* 1986; **90**: 5816.
40. Bensebaa F, Zhou Y, Brolo AG, Irish DE, Beslandes Y, Kruus E, Ellis TH. *Spectrochim. Acta, Part A* 1999; **55**: 1229.
41. Krolikowska A, Kudelski A, Michota A, Bukowska J. *Surf. Sci.* 2003; **532**: 227.
42. Mosier-Boss PA, Lieberman SH. *Spectrochim. Acta, Part A* 2005; **61**: 845.
43. Fleissner G, Kozlowski PM, Vargak M, Bryson JW, O'Halloran TV, Spiro TG. *Inorg. Chem.* 1999; **38**: 3523.
44. Michota M, Kudelski A, Bukowska J. *J. Raman Spectrosc.* 2001; **32**: 345.
45. Kudelski A. *Surf. Sci.* 2002; **502**: 219.