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Surface enhanced Raman scattering study of monolayers of 2-mercaptopurine at the gold surface

Xiaoyu Guo, Yali Pu and Haifeng Yang*

Department of Chemistry, Key Laboratory of Resource Chemistry of Ministry of Education, Shanghai Key Laboratory of Rare Earth Functional Materials, Shanghai Normal University, 100 Guilin Road, Shanghai 200234 People's Republic of China.

Dedicated to Professor Dr S Mohan

The monolayers of 2-mercaptopurine (2MP) were self-assembled at the roughened polycrystalline gold surface in acid and alkaline media. The interpretation of SERS mapping spectral data aided by the calculations for the vibrational modes using BLYP/6-31G method suggested that the resulted 2MP monolayers under acid condition through the S and N_3 atoms of pyrimidine moiety anchoring at the gold surface in a tilted fashion and in basic solution, formation of 2MP molecules by the interaction of the C=S bond and N_1 atom with the surface. In-situ SERS spectroelectrochemical experiment was conducted to examine the stability of the SAMs, and it was found the detaching process of the 2MP SAMs formed under acid media had happened around -1.6 V vs SCE. The monolayers of 2MP formed at pH 13 were detached from the Au surface at ca. −1.2 V vs SCE.

Keywords: 2-mercaptopurine; SERS mapping; spectral assignments.

1 Introduction

Thiopurines as a kind of the clinically significant drugs can destroy cancer cells by interfering with a specific phase of the cells' life cycle as well as are commonly developed as anti-leukemic drugs or as anti-inffiammatory gastrointestinal and immunosuppressive agents [1-5]. Thiopurines have been modified at the metallic electrode as a well-defined functional surface to promote the electron transfer process of some proteins [6-9]. As the above stated attractive applications, the thiopurines have been extensively investigated by electrochemically and spectroscopically analytical methods.

At the silver electrode, a striping voltammetric study for the electrochemical features of thiopurines including 6-mercaptoguanine (6-MG), 6-mercaptopurine (6-MP), 6-thioxanthine, and 2-thioxanthine and 2-mercaptopurine (2-MP) have been performed by Zeng and Purdy [10,11]. Madueño *et al* [12,13] have reported the voltammetric behavior of 6-MP monolayers on Hg and Au surfaces, and proposed that the 6-MP molecules are attached at the gold surface via N_7 and S atoms; while in the case of Hg the adsorption mode involved N_1 and S atoms in a basic media.

Taniguchi *et al* [8] found that the 6-MP modified gold electrode can promote the electron transfer process of cytochrome C, and suggested that 6-MP adsorb onto the Au surface via the S atom in $S{=}C_6$ and N_7 with a vertical orientation under the applied potential at 0 V vs SCE. Hosten and co-workers [14] reported that 6-MP attaches head-on through the N_1 atom with respect to the Ag surface on the basis of the surface enhanced Raman scattering (SERS) data obtained in a slightly basic 6-MP solution over the surface. We have conducted a time-dependent Raman mapping spectral analysis in conjunction with the vibrational calculations using *ab-initio* BLYP/6-31G to find out that from both acidic and basic solutions the 6-MP molecules eventually formed monolayers at the Au surface through the S atom of pyrimidine moiety and N_7 atom of imidazole moiety in a vertical way [15].

Up to date, there is no literature about the interaction between the 2-MP molecules and the metallic surface at molecular level, inspite of some reports on the electrochemical behavior of 2-MP molecule different from that of 6-MP at the Ag electrode surfaces by Zeng and Purdy [11]. Further investigation is desired to shed light on the adsorption mode of 2MP molecules at the metallic surface.

Corresponding author :
e-mail: *haifengyang@yahoo.com*; *Tel.: Fax: +86 021 64322511* (Haifeng Yang)

In this study for the first time, we extend the above-mentioned SERS mapping to observe the adsorption procedure of the 2MP monolayers at the Au surface in acid and alkaline media. The use of a microscope as an integral part of the SERS optical system could provide relatively precise tool to do mapping the interface in two dimensions for understanding the feature of the resulted films [16]. The structural properties of two kinds of 2MP self-assembled monolayers (SAMs) on Au electrodes were based on the assignments of the vibrational modes, and their electrochemical stabilities were examined by in situ SERS spectroelectrochemical measurements.

2 Experimental and Methods

2.1 Chemicals

2-mercaptopurine monohydrate (95 %) was purchased from Sigma and used as received. Two stock solutions of 10^{-1} mol/L 2MP were prepared with dissolution in 0.01 mol/L HCl and 0.01 mol/L KOH solutions, respectively. Doubly-purified water was freshly prepared and bubbled with nitrogen for ~15 min to remove oxygen. All other reagents were of analytical grade.

2.2 Roughening the surface of gold electrode

The Au electrode was constructed from polycrystalline material (99.99 % pure). Before Raman measurement, the Au electrode was polished successively with emery paper, 1, 0.3 and 0.05 μm alumina, and then treated in a 0.1 mol/L KCl solution by an ORC method [17]. The Au electrode after washing with doubly-purified distilled water was put into a home-made electrochemical cell with a quartz window at the top. During this pretreatment and *in-situ* spectroelectrochemical measurements, the experiments were conducted by CHI 760B electrochemical workstation. Pt wires served as counter electrodes, and all the potentials were quoted versus a standard calomel electrode (SCE).

2.3 Self-assembled monolayers

After the cell was installed onto XY stage below the sampling objective of Raman spectroscopy, the 2MP solution freshly diluted into 10^{-4} mol/L was injected into the cell for self-assembling monolayers at the roughened Au surface and the procedure was investigated by Raman mapping measurement.

2.4 Raman mapping and in-situ SERS spectroelectrochemical measurement

Raman spectra were recorded with Jobin Yvon Micro-Raman Spectrometer (RamLab-010). It comprises an integral Olympus BX40 microscope with a 50× objective (8 mm), which can focus the laser on the sample to collect the back scattered radiation, a notch filter to cut the exciting line, a holographic grating (1800 g/mm), which provides a spectral resolution of 2 cm^{-1}, and a semiconductor- cooled 1024×256 pixels CCD detector. A laser of 632.8 nm with a power of ca.5 mW as excitation line and a spot of ca.3 μm in diameter on the electrode surface were used. The slit and pinhole were respectively set at 100 μm and 300 μm in confocal configuration to increase the spatially spectral resolution. Collection time was of 30 s with 2 times accumulation for each spectrum. An XY stage with the capability of one moving step for 1 μm controlled by PC was utilized to perform Raman mapping. The mapping area of *ca.* 70×40 μm^2 was squared randomly on a monochrome image of the surface by the use of the optical axis conjugate camera and a white light source. Calibration was done referring to the 520.6 cm^{-1} line of silicon.

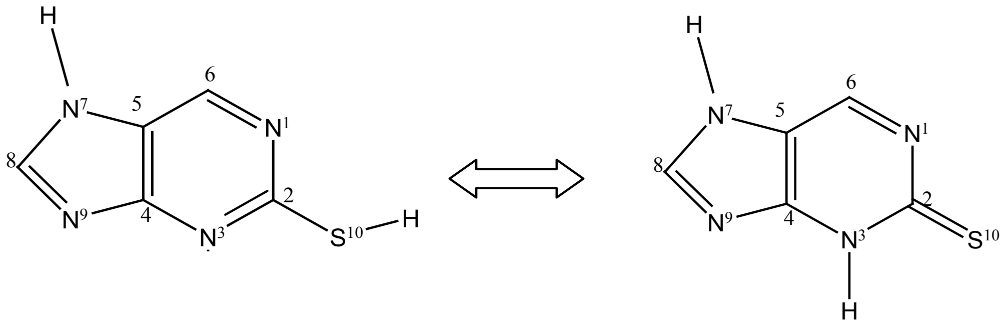
2.5 Theoretical Methods

Full geometry optimization and computation of vibrational modes of 2MP were performed by BLYP /6-31G method [18, 19] in Gaussian 98 software.

3 Results and Discussion

3 Raman mapping of 2MP SAMs at the Au surface in acid media

As an aromatic thiol molecule 2MP possesses possible sites such as S_{10}, N_9, N_7, N_3 or N_1 to attach the metallic surface (see scheme1), SERS is a powerful spectroscopic technique able to provide rich molecular level information for the elucidation of binding, orientation and conformation of adsorbed molecules at interfaces due to its high sensitivity, special surface selection rules and mechanisms especially for the chemical or electronic enhancement regarded as an increase in Raman scattering intensity in the first monolayer.



Scheme 1. Structures of 2-mercaptopurine in thiol form and its tautomer in acid media

The SERS mapping spectra of 2MP molecules adsopbed at the Au surface in the acid solution was recorded and displayed in Fig 1. It is clearly seen that, all SERS spectral features in the frequency of the mapping profiles are identical, indicating the 2MP molecules self-assembled at the Au surface in a same adsorption mode to form monolayers with uniformity and order state. Additionally, the quite variation of the intensities of speactral curves obtained from the different spots of surface in Fig 1 could be interpreted as the impact of the varying roughness of the Au surface by point and point on SERS signals.

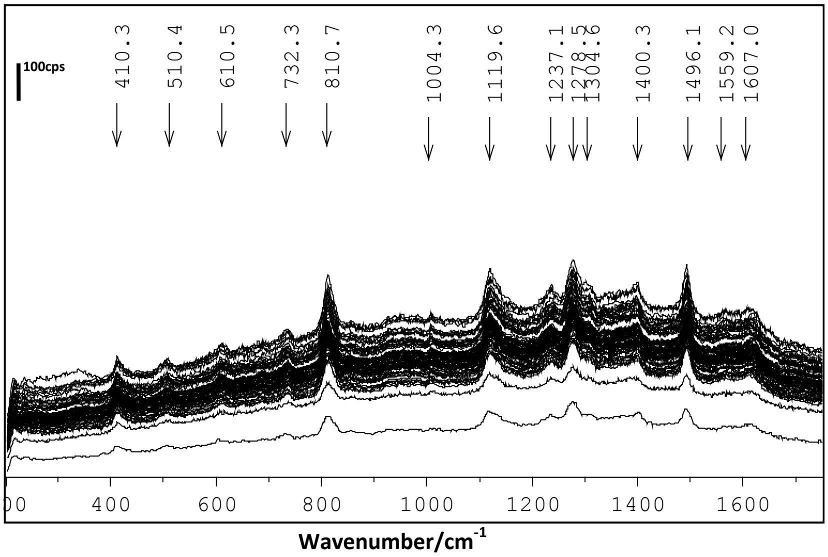


Fig 1. SERS mapping of 2MP monolayers at the roughened Au surface in an acid media

From the observation in Fig 1, the main peaks appear around 410, 510, 610, 732, 810, 1119, 1237, 1278, 1304, 1400, 1496, 1559 and 1607 cm^{-1} by surface enhancement. Assignments of these SERS bands were made by comparison with BLYP /6-31G calculation and summarized in Table 1.

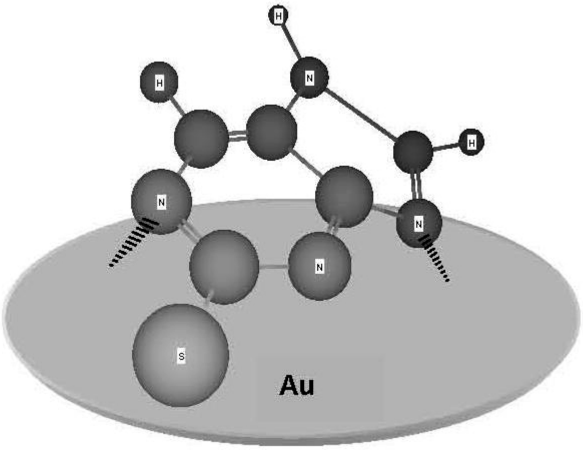
Table 1. Assignments for the observed SERS bands of 2MP molecule at the roughened Au surface under acid condition (wavenumber in cm^{-1})

Raman/cm^{-1}	BLYP/6-31G	Approximate assignment
410(m)	444	Two p(pyrim (N3))+δ(C6-H)
610(w)	594	br (pyrim (N3))
732(w)	714	δ_{op}(C2-S)+Two p (purine)
	732	δ(S-H)+Tw(purine)
810(s)	801	br(pyrim) +δ (S-H)
1004(vw)	1106	v (N_7-C_8)
1119(s)	1151	δ (C_4-N_3-C_2)+v (C_2-N_1)+δ (C_8-H)+δ (C_7-H)
1237(m)	1223	δ (C_4-N_3-C_2)+v (C_2-N_1)+ δ (C_8-H)+ δ (C_6-H)
1278(s)	1253	v (C_4-N_3-C_2)+v (C_2-N_1)+δ (C_8-H)
1304(w)	1306	v (N_9-C_4)+v (N_1-C_6)+δ (C_8-H)+δ (N_7-H)+ δ (C_6-H)
1400(w)	1402	v (N_9-C_4-C_5)+v (N_3-C_2)+δ (C_8-H) +δ (N_7-H)+ δ (C_6-H)
1496(s)	1507	v (C_8-N_3)+v (C_6-C_9)+δ (C_8-H) +δ (N_7-H)+ δ(C_6-H)
1559(sh)	1574	v (C_5-C_4-N_3)+v (C_2-N_1-C_6)+δ(N_7-H)
1607(w)	1649	v (C_4-N_3-C_2)+v (C_6-C_5) +δ (C_8-H)+δ (N_7-H)

*The scale factor was of 0.994
v;stretching vibration, δ; deformation vibration, br; ring breathing vibration, op, out-of-plane
Pyrim; Pyrimidine, sh; shoulder peak, vw; very weak , s; strong, m; medium, w;weak.

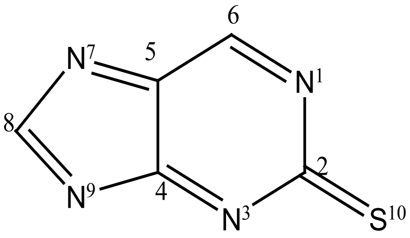
The band of the S-H vibration near 2530 cm^{-1} is unobservable (the SERS spectral range from 1700 to 3300 cm^{-1} not shown in Figure 1) and the week band at 732 cm^{-1} corresponding to the C_2-S deforming mode still presents. These observations suggest that 2MP should adsorb on Au surface via the S atom upon the cleavage of the S-H bond, leading to the occurrence of the bands at 410 and 610 cm^{-1} resulting from deformation vibration of the N_3 atom of the pyrimidine ring along with the breathing vibration of this ring. The bands at 1119 and 1237 cm^{-1} are due to the mixture of C_4-N_3-C_2 deforming and C_2-N_1 stretching vibrations. The C_4-N_3-C_2 stretching vibration occurs around 1278 cm^{-1}, while the band at 1496 cm^{-1} assigned to the vibration of C_8-N_3. It has been generally accepted that the observed SERS signals mainly resulted from the constituent chemical moieties of the molecule close to or direct adsorbed on the SERS-active surface [20]. Therefore, besides S atom the pyrimidine ring attached onto the Au surface through N_3 atom involving N_1 atom also very closer to the surface could be deduced. The present of the week bands at 1400 and 1304 cm^{-1} related to the vibrational modes of the N_9-C_4 on the imidazole ring depicted the N_9 atom also approaching the Au surface. In a conclusion, 2MP molecule might adopt a tilted orientation on the Au surface under acidic condition via S and N_3 atoms, and the N_1 and N_9 atoms might both contribute to this kind of attachment. The adsorption mode was displayed in Scheme 2.

It should be mentioned that the vibration modes of 2MP tautomer have been also estimated in view of the results of BLYP/6-31G calculation, which predict the appearance of the N_3-H deforming vibrations of 2MP near 1378 and 1524 cm^{-1} but the bands both absent in the experimental SERS date. In addition, based on the MP3 minimized energy calculation, the molecular energy of 2MP in thiol form is –1487.852 eV lower than that of its tautomer with –1487.301 eV as a stable existing fashion. Accordingly, 2MP in thiol form might be naturally selected to self-assemble the monolayers after the cleavage of S-H bond.



Scheme 2. A plausible adsorption mode of 2MP monolayers at the Au *surface in the acid solution*

3.2 Raman mapping of 2MP SAMs at the Au surface in basic media



Scheme 3. Possible form of 2MP molecules in the strong alkaline solution

As shown in Scheme 3, there is the dominating structure of 2MP molecule after completely deprotonating of S-H and N_7-H in the strong alkaline solution with the pH 13. The resulted SERS mapping spectra of 2MP monolayers formed under basic media are presented in Figure 2. The tentative attributions to the SERS bands together with the vibrational predication by BLYP /6-31G are tabulated in Table 2.

Table 2. Assignments for the observed SERS bands of 2MP molecules at the Au roughened surface under basic media (wavenumber given in cm^{-1})

Raman cm^{-1}	BLYP/6-31G	Approximate assignment
408(m)	417	br(pyrim)
734(w)	711	Tw_{op} (pyrim)
802(s)	794	Tw_{op} (pyrim)+ δ(C8-H)
825(s)	811	δ(C4+N3+C2)+δ(N1-C6-C5)+δ(C8-H)
854(w)	850	δ(C2-N1-C6)+δ(C6-H)+ v (N9-C4)
1126(s)	1088	v (C2=S) +δ(C4-N3-C2 -N1)
1226(s)	1213	v (C2=S)+ δ(C8-H) +δ(N3-C2-N1)
1283(sh)	1307	δ(C8-H) + δ(C6-H)
1306(s)	1357	δ(C6-H)+δ(C8-H) +d(N1-C6-C5)
1402(m)	1370	δ(C6-H))

1454(m)	1473	δ(C8-H) +*v* N9-C4-N3)
1491(m)	1531	*v*(N1-C6)+δ(C6-H)+δ(C8-H)

*The scale factor was of 0.994
v;stretching vibration, δ; deformation vibration, br; ring breathing vibration, op, out plane
Pyrim; Pyrimidine, Imid;Imidazole, sh; shoulder peak,,s; strong, m; medium, w;weak.

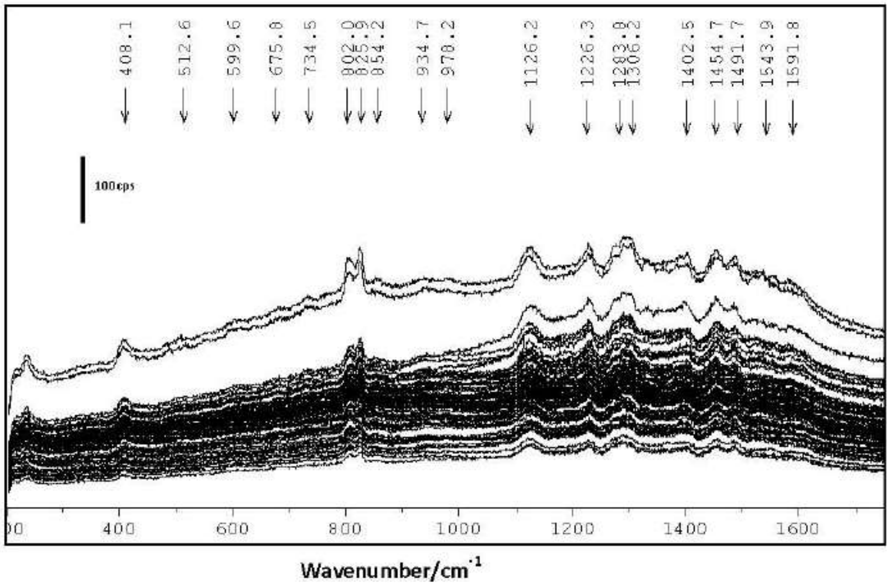
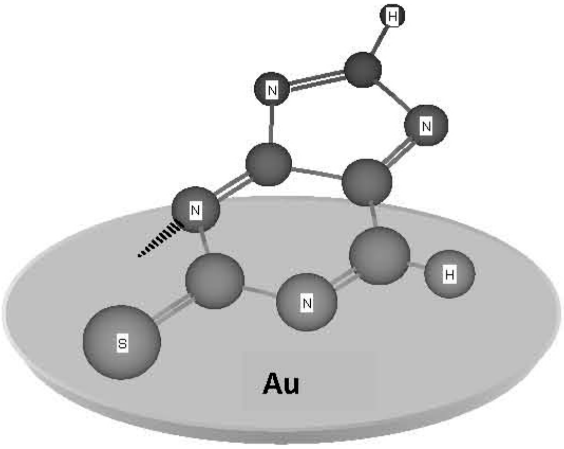


Fig 2. SERS mapping of 2MP SAMs at the roughened Au surface within the basic solution

It could be found that the much stronger bands observed in Fig 2 are relative to the vibrational modes of C=S, N_1-C_2-N_3 and N_1-C_6-C_5 located around 1126, 1226 and 1306 cm^{-1}. Furthermore, considering the occurrence of SERS bands assigned to breathing vibrations of pyrimidine ring, we suggested that the C=S bond and N_1 atom of the 2MP molecules under basic media anchor at the Au surface to organize the stable monolayers in a tilted fashion as well as the N_3 atom leading closer to the surface. A presumed mode of the 2MP molecules self-assembled at the Au surface from alkaline solution is shown in Scheme 4.



Scheme 4. A suggested adsorption mode of 2MP SAMs at the Au surface with the basic solution

3.3 In situ SERS Spectroelectrochemical measurements

After removing the 2-MP solution from the cell and washing the Au electrode with doubly purified water, a 0.1 mol/L KCl solution was injected into the cell as the supporting electrolyte with a very thin layer *ca* 0.1 mm over the electrode surface. In situ SERS spectroelectrochemical technique was then carried out to examine the stability of the 2-MP monolayers on the Au electrode by changing the applied potentials.

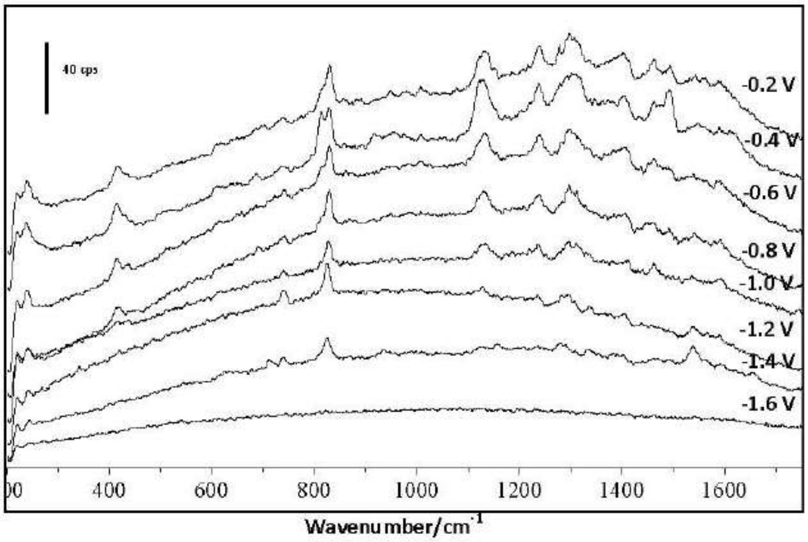


Fig 4. In-situ SERS spectra of the 2-MP monolayers formed on the Au surface under acid media recorded in a 0.1 mol/L KCl solution with the shift of the applied voltages vs SCE

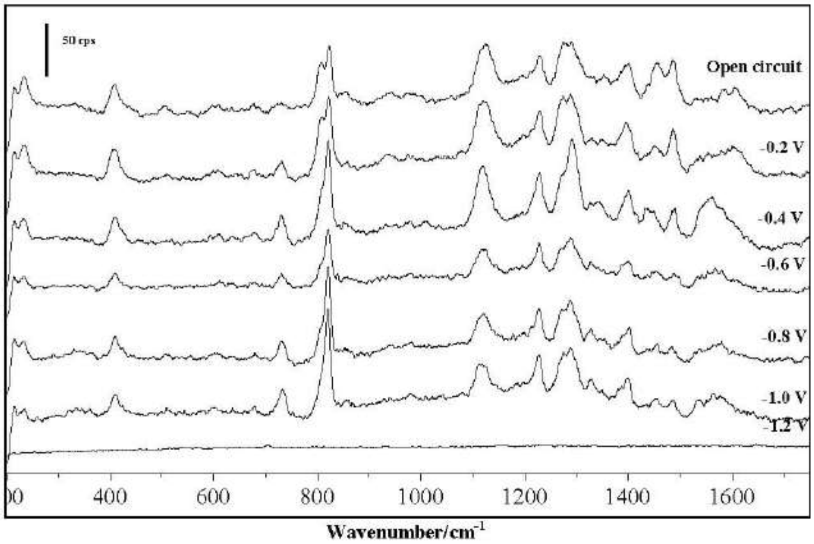


Fig 5. In-situ SERS spectra of the 2MP monolayers formed on the Au surface under basic media recorded in a 0.1 mol/L KCl solution with the shift of the applied voltages vs SCE

In Figs 4 and 5, we have shown the in situ SERS spectroelectrochemical spectra of 2-MP monolayers formed on the Au electrodes in acid and basic solution. During negatively moving the applied potential, both

SERS spectral features in frequencies are varying slightly in an exception of the vibration involving pyrimidine ring. However, in Figs 4 and 5, it could be observed that the desorption potential of 2-MP monolayers formed at the Au surface in acid solution around –1.6 V vs SCE are much more negative than that formed in basic solution at about -1.2 V *vs* SCE, which imply the stability of former monolayers self-assembled through Au-S covalence bond is much great over the later formed by the interaction between C=S bond and Au surface.

4 Conclusion

The formation of 2-MP monolayers on the Au electrode has been studied in acid and alkaline media by SERS mapping technique and the corresponding molecular adsorption fashion on the Au surfaces was deduced by interpretation of SERS bands on the basis of the calculation results by BLYP/6-31G and SERS producing mechanism. The electrochemical stability of the 2-MP monolayers on the Au electrode has been investigated by the use of *in situ* spectroelectrochemical measurements. Following conclusions could be reached:

(1) The self-assembling of 2-MP tends to attach directly onto the Au surface in acid media at an angled orientation through C-S and N_3 atom companying the N_1 and N_9 atoms co-contributions, whereas the sites of interactions with gold surface under basic condition should be the C=S and the N_1 atom of the pyrimidine moiety with the N_3 closer to the surface.

(2) The desorption of 2-MP SAMs formed under acid media has happened as the potential is applied to −1.6 V vs SCE. The monolayers of 2-MP formed at pH 13 are detached from the Au surface at the potential of −1.2 V vs SCE. The former monolayers are much electrochemical stability.

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