Surface-enhanced Raman scattering of 5-fluorouracil adsorbed on silver nanostructures

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Received 3rd March 2009, Accepted 30th April 2009 First published as an Advance Article on the web 10th June 2009 DOI: 10.1039/b903823j

Raman and surface-enhanced Raman scattering (SERS) of 5-fluorouracil (5-FU) have been recorded under several experimental conditions. SERS spectra have been analysed according to a resonant charge-transfer (CT) mechanism similar to a resonance Raman (RR) process, involving the photoinduced transfer of an electron from the Fermi level of the metal to vacant orbitals of the adsorbate (SERS-CT). In order to detect the enhancement mechanism and to identify the chemical species that give rise to the spectra, the theoretical SERS-CT intensities for the dienolic and diketo forms, and its respective N1 and N3 deprotonated anions (5-FU⁻), have been calculated and compared with the experimental results. In this way, the presence of N1 deprotonated anion is confirmed by SERS given that the calculated SERS-CT intensities predict the selective enhancement of the band at *ca*. 1680 cm⁻¹ in agreement with the experiment. Therefore, the metal-to-adsorbate CT process involves the transient formation of the respective radical dianion (5-FU^{\bullet -7}), which is new evidence of the relevance of the CT enhancement mechanism in SERS.

1. Introduction

5-Fluorouracil (5-FU) is interesting in pharmacology and biochemistry given that it belongs to the 5-halouracil chemotherapy drugs that prevent cell growth through its incorporation into DNA and RNA molecules, causing improper replication of nucleic acid bases and incomplete biochemical synthesis.¹ Additionally, these compounds in which the C5 atom is bound to halogen atoms are presently tested against HIV and used as antitumor and antiviral drugs.^{2,3}

Moreover, the adsorption of biomolecules on metal substrates is an interesting subject due to its broad potential application in environmental and biological trace analysis.^{4,5} In this respect, surface-enhanced Raman scattering (SERS) is a very powerful technique in order to gain insight into the nature of metal–adsorbate interactions, namely the bound molecular species and its orientation with respect to the metal nanostructures.⁶ Thanks to its very high sensitivity, SERS is used nowadays as a routine analytical tool acting like a chemical sensor and, in favourable cases, it is even able to detect the Raman signal arising from single molecules.^{5,7}

Many of the published works on SERS^{8–10} deal with aromatic molecules and they are usually interpreted on the basis of the selection rules derived from the electromagnetic enhancement mechanism (EM). However, this mechanism is not able to explain the selective enhancement of certain bands of the same symmetry or the role of the electrode potential in the relative intensities. This complex behaviour is often due to the presence of the so-called charge-transfer (CT) enhancement mechanism. One of the most important open questions in SERS is indeed how to know which enhancement mechanism is acting in a particular record. This is a very old controversy and our research group has developed a methodology to detect the presence of CT processes by assuming that the SERS-CT enhancement mechanism is analogous to a Raman resonance (RR)¹¹ process where the incident photon ($h\nu$) produces the resonant transfer of one electron from the metal to vacant orbitals of the adsorbate (A). This yields, from the adsorbate point of view, the transient formation of the corresponding radical anion (A^{•-}) of neutral molecules in the excited CT state:

$$A + e^- + h\nu \to A^{\bullet^-}$$

When the electron comes back to the metal, a Raman photon is emitted $(h\nu')$ if the molecule remains vibrationally excited (A*):

$$A^{\bullet -} \rightarrow A^* + h\nu' + e^-$$

This CT mechanism allows us to predict the selective enhancement of the bands following the formalism for RR by Albrecht *et al.*¹¹ On the basis of this methodology, the selective enhancement observed in SERS of different aromatic molecules such as pyrimidine,¹² pyridine¹³ and pyrazine,¹⁴ and their analogues^{15,16} has been explained. In conclusion, the main feature observed in the SERS-CT of these aromatic molecules is the strong enhancement of the band corresponding to the 8a mode recorded at about 1600 cm⁻¹.

In this work, the SERS spectrum of 5-FU adsorbed on silver sol and electrode has been recorded and analysed assuming the presence of the SERS-CT enhancement mechanism. This is a

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Fig. 1 B3LYP/6-31G* relative energies between the diketo and dienol forms of 5-FU and the N1 and N3 deprotonated anions corresponding to the diketo form, N1 and N3, respectively.

rather complex system given that different molecular species or ionic forms can be present in aqueous solution^{17,18} and, therefore, another challenge is to recognize which of them gives rise to the SERS. Under different pH values several chemical species of 5-FU can exist. In order to simplify the problem, the discussion will be restricted to four of them, namely the two tautomeric forms related by a keto–enolic equilibrium (diketo and dienolic) and two anions of the diketo form, N1 and N3, respectively, yielded by the deprotonation of the respective nitrogen ring atoms (Fig. 1). In order to identify the chemical species that gives rise to the spectra, the SERS intensities under resonant CT conditions have been calculated for each one of these four species and compared with the experimental results.

2. Experimental

5-FU of the highest available purity (99%) has been purchased from Sigma. Raman spectra of the solid and of 0.5 M aqueous solution at pH = 7 and pH = 14 have been recorded by using the 1064 and 514.5 nm excitation lines, respectively. A 10^{-3} M aqueous solution of 5-FU has been used to record all SERS, and 0.1 M Na₂SO₄ has been used as electrolyte in the spectra recorded on a silver electrode. Triple distilled water was employed and the pH was adjusted by adding dropwise a NaOH solution.

Colloidal silver sols have been prepared according to the method described by Creighton *et al.*¹⁹ The complete procedure has been explained elsewhere.²⁰ Basically it consists of reducing an aqueous solution of 10^{-3} M AgNO₃ with an excess of 2×10^{-3} M NaBH₄. The proportion of the reagents AgNO₃–NaBH₄ was 1 : 3. After stirring the mixture it is allowed to rest at room temperature for about 90 min. Sometimes, during this waiting time, a dark colour in the heart of the solution appears, and at this moment vigorous stirring is necessary to stabilize the sol. This is a transparent, yellow solution, with a maximum in its absorption spectrum at 390 nm. An aqueous solution of adsorbate is then added to the colloid to obtain the desired concentration. At that moment a change in the colour of the system, from the initial yellowish to a final blue–greenish, is observed.

The experimental procedure for obtaining SERS spectra on an electrode has been detailed elsewhere.^{13,14,21} Briefly, the silver electrode is subjected to a careful electrochemical treatment to obtain the necessary surface roughness. First, it is polished with 1.00, 0.30 and 0.05 μ m alumina (Büeler) and then electrochemically activated by maintaining the electrode

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potential at -0.50 V and then subjecting it to eight, 2 s pulses at +0.60 V. During this procedure, a 0.1 M aqueous KCl solution was employed as the electrolyte. A Princeton Applied Research (PAR) model 173/175 potentiostat/programmer was used to control the three-electrode cell fitted with a platinum counter electrode, a saturated Ag/AgCl/KCl reference electrode, and the pure silver working electrode.

The Raman and SERS spectra were recorded using a Jobin-Yvon U-1000 double monochromator spectrometer fitted with a cooled Hamamatsu R943-02 photomultiplier, using the 514.5 nm exciting line from a Spectra Physics 2020 Ar^+ gas laser. A constant slit width was used that allowed a spectral resolution of 4 cm⁻¹; the laser power reaching the sample was kept at 500 mW. The band wavenumbers were measured with the help of the same laser plasma lines as standards, whereby a precision of ± 2 cm⁻¹ was obtained under the employed operating conditions. The FT-Raman spectrum of the solid was also recorded on a RFS-100 Bruker FT-spectrometer, fitted with a Nd:YAG laser using the 1064 nm excitation wavelength and the laser power set to 400 mW. A few milligrams of the sample were placed into a small aluminium sample cup and lightly packed. The spectrum



Fig. 2 (a) FT-Raman spectra of solid 5-FU (excitation line $\lambda = 1064$ nm); Raman spectra (excitation line $\lambda = 514.5$ nm) of 0.5 M aqueous solution of 5-FU at (b) pH = 7, (c) pH = 10 and (d) pH = 14 and SERS spectra on silver sol of 10^{-3} M solution at (e) pH = 7 and (f) pH = 12.

corresponds to the average of two repeated measurements of 100 scans each and 2 cm⁻¹ resolution.

In order to assign the spectra, the geometrical structure and the force field of the different chemical species of 5-FU have been calculated at the B3LYP/6-31G* level of theory. The calculated harmonic frequencies are scaled by 0.9614 as usual to account for anharmonicity and electron correlation effects.²² In previous works, it has been demonstrated that this level of calculation reproduces satisfactorily the vibrational spectrum of uracil.²³ All calculations have been carried out with the GAUSSIAN 03 program package.²⁴

3. Results and discussion

Raman spectra of solid and aqueous solution of 5-FU

Geometrical optimization of neutral 5-FU at the B3LYP/ 6-31G* level of theory yields a planar structure. All the calculated wavenumbers are real indicating that this structure is a minimum. Several theoretical studies on uracil^{25,26} point out that the diketo form is the most stable tautomer, the dienolic one being *ca*. 10–25 kcal mol⁻¹ less stable. The calculated B3LYP/6-31G* energy difference between both neutral forms amounts to 15.5 kcal mol⁻¹ yielding the diketo more stable than the dienolic one in agreement with the mentioned studies on uracil. Therefore, the Raman frequencies of the solid have been correlated with those calculated for the diketo form. Fig. 2 shows the Raman spectrum of solid 5-FU and Table 1 collects the corrected B3LYP/6-31G* wavenumbers and the assignment of the bands according to the results for this form. Generally speaking, the vibrational assignment of the Raman spectrum of 5-FU is similar to that proposed for uracil.^{27,28} The spectrum of the solid is characterized by a strong band at 1349 cm⁻¹, which can be assigned to $\delta(N_1$ -H) or $\delta(N_3$ -H) modes, and two medium bands recorded at 1225 and 769 cm⁻¹ and assigned to $\nu_{\text{ring}} + \nu(C_5$ -F) and ring breathing modes, respectively. The strong band recorded at 112 cm⁻¹ is attributed to a lattice vibration.

Fig. 2 shows also the Raman spectra of 5-FU aqueous solution at pH = 10 and 14, respectively. The assignment of these spectra is also collated in Table 1 together with the calculated frequencies for both deprotonated N1 and N3 anions. The pH values have been chosen according to the pK_a of 5-FU. The first one ($pK_{a1} = 8.15$) is lower than in uracil ($pK_a = 9.45$) as expected taking into account the electronegativity of fluorine and should be assigned to the ionization of the N1–H bond given that the corresponding anion is more stable than the N3 one (Fig. 1). No data concerning the second pK_{a2} , corresponding to the formation of the dianion, have been found in the literature, but it is expected to be lower than in uracil ($pK_{a2} = 13$).¹⁸

Although the N1 anion should be the major species in solution at pH = 10, the Raman spectra indicate that the N3 deprotonated form could also be present, reaching a larger concentration at pH = 14. The Raman spectra of both

Raman solid	Diketo form	Assignment	Raman pH = 10	N3 anion	Raman pH = 14	N1 anion	Raman pH = 7
	3503	ν (N ₁ -H)	_	3478	_	_	_
	3461	$\nu(N_3 - H)$	_	_	_	3457	_
	3126	$\nu(C_6-H)$	_	3072	_	2982	_
1671	1777	$\nu(C = O)$	1679	1688	1680	1687	1675
1648	1745	$\nu(C=O)$	1621	1614	1609	1670	1617
1506	1668	$\nu_{ring} + \nu(C = C)$	1531	1669	1547	1566	1535
1426	1454	ν_{ring}	_	1411	_	1429	1409
1349	1382	$\delta(N_3 - H)$	1354		1356	1323	1346
	1355	$\nu_{\rm ring} + \delta({\rm N_1-H})$	1337	1300	1330	_	_
1258	1308	$\delta(C_6-H) + \nu_{ring}$	1290	1242	1284	1273	1280
1225	1236	$\nu(C_5-F) + \nu_{ring}$	1232	1270	1210	1260	1246
1186	1146	$\delta(C-H) + \delta(N_1-H)$	1187	1091	1187	1168	1204
_	1120	ν_{ring}	_	1158	_	1080	_
996	934	12; δ_{ring}	1014	895	1024	925	1036
810	854	$\gamma(C_6-H)$	822	810	816	919	834
	786	$\delta_{ring} + \nu(C_5 - F)$	_	777	_	786	_
	719	$\gamma(C_2 = O) + \gamma(C_4 = O)$	_	732	_	744	_
769	717	1; ν_{ring}	771	703	783	724	794
	713	$\gamma(C_2 \equiv O) + \gamma(C_4 \equiv O)$	_	708	_	706	_
	650	$\gamma(N_3-H)$	_		_	643	_
639	604	$\delta(C_2 = O) + \delta(C_4 = O)$	647	606	655	615	655
546	515	δ_{ring}	573	521	581	540	583
	524	$\gamma(N_1 - H)$	_	479	_	_	_
471	437	δ_{ring}	489	453	499	439	495
367	373	$\delta(C_2 = 0) + \delta(C_4 = 0)$	364	397	362	371	372
	362	τ _{ring}	_	328	_	425	_
	332	τ_{ring}	_	260	_	307	_
	288	$\delta(C_5 - F) + \delta(C_4 = O)$	_	287	_	282	_
167	146	τ_{ring}	_	165	_	159	_
	112	$\gamma(C_5 - F) + \gamma(C_2 = O)$	_	56	_	119	_
112	—	Lattice vibration		—	—	_	—
ν , stretching; δ	, in-plane bendi	ng; y, out-of-plane deform	ation; τ , torsion.				

aqueous solutions are characterized by a strong band recorded at about 770–780 cm^{-1} which is assigned to the ring breathing vibration. However, a different behaviour is found in the 1300 cm^{-1} region. At pH = 10, it can be seen that the band recorded at 1354 shows a shoulder at 1337 cm^{-1} . On the basis of the calculated force fields these two bands are assigned to N₃-H and N₁-H in-plane deformations of the respective N1 and N3 anions. Both bands are also observed in the Raman at pH = 14, but in this case the intensities are interchanged. That recorded at 1330 cm^{-1} , stronger than that at 1356 cm^{-1} , should be assigned to the N₁-H in-plane deformation. Therefore, these two strong bands at 1354 and 1330 cm^{-1} can be used as markers for the N1 and N3 deprotonated anions, respectively. Although it is not possible to determine the N1 to N3 ratio since the spectra of the pure deprotonated ions are not available, it can be estimated to be 2:1 and 1:4 at pH = 10and 14, respectively, by taking into account the relative intensity of both bands. This result indicates that the N3 deprotonated form is present at pH = 7 and predominates at pH = 14 as it has been found in the solutions of uracil.¹⁸ Summarily, the N1 form slightly predominates at pH = 10, while at pH = 14 the N3 form is the major form. No evidence of the dianion has been found at any pH value. Finally, it is to be stressed that the Raman wavenumbers of the aqueous solutions are blue-shifted up to 30 cm⁻¹ with respect to those of the solid due to the effect of hydrogen bonding between 5-FU and water molecules as occurs also in uracil.²⁹ For instance, the band recorded at 546 cm⁻¹ and assigned to δ_{ring}

in the solid is recorded at 573 cm^{-1} in the spectrum of the solution at pH = 10.

SERS spectra

SERS spectra of 5-FU on silver sol at neutral and alkaline pH are shown in Fig. 2. It can be observed that the spectrum at neutral pH is characterized by three strong bands recorded at 794, 1346 and 1675 cm⁻¹ assigned to in-plane modes. The most important difference between the Raman and SERS spectra is the selective enhancement of the last band. Table 2 collects the SERS wavenumbers together with the vibrational assignment. It can be seen that SERS wavenumbers correlate quite well with those of the aqueous Raman spectrum; the shifts are not very large and are due to the effect of the adsorption of 5-FU on the metal surface.¹² At alkaline pH the behaviour is slightly different. The strongest band is that recorded at 800 cm⁻¹, while that at 1673 cm⁻¹ does not show the selective enhancement observed in the SERS at pH = 7.

All SERS bands at neutral pH are also observed at pH = 12 and, therefore, the adsorbed species does not change with the pH. Only one band is recorded in both SERS in the 1300 cm⁻¹ region, which could be alternatively assigned to either $\delta(N_1$ -H) or $\delta(N_3$ -H); therefore, it is not possible to identify the chemical species that gives rise to the SERS. However, given that the stronger SERS bands correspond to in-plane modes, no out-of-plane modes are observed, and the C=O stretching vibration at 1675 cm⁻¹ is recorded,

B3LYP/6-31G*		Raman	SERS sol		SERS electrode		
Diketo form	Assignment	pH = 10	pH = 7	pH = 12	0.0 V	-0.25 V	-0.50 V
3503	ν (N ₁ -H)				_		_
3461	ν (N ₃ -H)	_	_	_		_	_
3126	$\nu(C_6-H)$	_	_	_		_	_
1777	$\nu(C=0)$	1679	1675	1673	1669	1671	1671
1745	$\nu(C=O)$	1621	1617	1617	1613	1619	1617
1668	$\nu_{\rm ring} + \nu(C=C)$	1531	_	1567	1529	1533	1539
1454	$\nu_{\rm ring}$	_	1409	1425	1419	1413	1414
1382	$\delta(N_3-H)$	1354	1346	1340	1346	1344	1346
1355	$\nu_{\rm ring} + \delta(N_1 - H)$	1337	1346	1340	1346	1344	1346
1308	$\delta(C_6-H) + \nu_{ring}$	1290	1280	1286	1280	1276	1274
1236	$\nu(C_5-F) + \nu_{ring}$	1232	1246	1228	1240	1238	1238
1146	$\delta(C-H) + \delta(N_1-H)$	1187	1204	1202	_	_	1196
1120	$\nu_{\rm ring}$	_	_	_		_	
934	12: δ_{ring}	1014	1020	1050		1024	1022
854	$\gamma(C_6 - H)$	822	834	834	828	826	824
786	$\delta_{\rm ring} + \nu(\rm C_5-F)$			_		_	
719	$\gamma(C_2 = 0) + \gamma(C_4 = 0)$	_		_		_	_
717	1: ν_{ring}	771	794	802	786	785	783
713	$\gamma(C_2 = 0) + \gamma(C_4 = 0)$			_			
650	$\gamma(N_3-H)$	_		_		_	_
604	$\delta(C_2 = O) + \delta(C_4 = O)$	647	650	650	655	653	663
515	δ_{ring}	573	583	580	589	579	575
524	$\gamma(N_1-H)$			_		_	
437	δ_{ring}	489	495	507		_	
373	$\delta(C_2 = 0) + \delta(C_4 = 0)$	364	372	374		_	
362	τ_{ring}	_	_	_		_	
332	τ_{ring}	_		_		_	_
288	$\delta(C_5 - F) + \delta(C_4 = O)$	_		_	268	268	268
146	τ_{ring}	_		_			
112	$\delta(C_5 - F) + \delta(C_2 = O)$	—		—	—		—
ν , -stretching; δ , in	-plane bending; γ, out-of-plane	deformation; τ , t	orsion.				

Table 2 Scaled B3LYP/6-31G* and SERS wavenumbers (cm⁻¹) of 5-FU on silver sol and on silver electrode at different potentials



Fig. 3 (a) Raman spectrum of 0.5 M aqueous solution at pH = 10; SERS records on silver at electrode potentials (b) 0.00 V, (c) -0.25 V, and (d) -0.50 V obtained from an aqueous solution of 0.1 M in Na₂SO₄ and 10⁻³ M in 5-FU at pH = 7.

it can be deduced that 5-FU adsorbs on silver through one of the deprotonated nitrogen atoms adopting an almost perpendicular orientation.

The SERS of 5-FU recorded on silver at neutral pH and different electrode potentials are collected in Fig. 3. These spectra show similar behaviour to those recorded on silver sols. All spectra are characterized by three very strong bands recorded at 780, 1334 and 1671 cm^{-1} . When the electrode potential becomes more negative, it can be seen that the intensity of the 780 cm^{-1} band increases, reaching at -0.50 V an intensity similar to that recorded in the Raman of the aqueous solution. On the contrary, the intensity of the bands recorded at 1344 and 1670 cm^{-1} remains constant under different potentials. Once again, only one band is observed at 1344 cm⁻¹, therefore, it is not possible to decide which form gives rise to the spectrum. The main difference when Raman and SERS spectra are compared is again the strong enhancement of the band recorded at 1675 cm⁻¹ and assigned to the ν (C=O) vibration.

SERS intensities and CT processes

In order to analyse the selective enhancement observed in SERS, the theoretical intensities for a photoinduced CT

mechanism have been calculated according to the equations given by Wolde *et al.*³⁰ for an RR process. In this case, the relative Raman intensity of a particular normal mode "*j*" under pre-resonance with an excited state can be estimated by using the equation:

$$I_i = \Re B_i^2 \omega_i^2$$

where ω_j is the wavenumber corresponding to the *j*-normal mode in the ground electronic state and \Re is a constant that can be arbitrarily adjusted to normalize the relative intensities. The B_j parameter is related to the differences between the equilibrium geometries of the molecule in both resonant states and can be obtained by computing the molecular forces (*f*) at the Franck–Condon point of the potential energy surface of the excited electronic state, *i.e.*, the corresponding radical if a CT process is involved. B_j is given by the expression:

$$B_i = (2.41 \times 10^6) f \sqrt{M} L_i \omega_i^{-3/2}$$

where L corresponds to the normal mode matrix of the ground electronic state and M is the matrix of atomic masses.

In the case of 5-FU, the SERS-CT intensities can be calculated for four possible CT processes involving the neutral or anionic forms:

Neutral molecule: $A + e^- + h\nu \rightleftharpoons A^{\bullet-}$ (for the diketo and dienol neutral forms).

Anion: $A^- + e^- + h\nu \rightleftharpoons A^{\bullet 2-}$ (for the N1 or N3 deprotonated anions).

Fig. 4 shows the calculated SERS-CT spectra corresponding to these four CT processes as well as the SERS on silver sol at pH = 7. The theoretical spectra have been drawn by putting Gaussian functions at the calculated wavenumbers with areas proportional to the calculated relative intensities. It can be observed that there are significant differences depending on the species involved. In conclusion, only the theoretical spectrum corresponding to the N1 deprotonated anion reproduces the experimental behaviour, i.e., the selective enhancement of the ν (C=O) mode at 1675 cm⁻¹. The spectrum calculated for the N3 anion is characterized by two medium bands at 1671 cm⁻¹ and 1687 cm⁻¹ showing similar intensities and assigned to $\nu(C=C)$ and $\nu(C=O)$ modes, respectively, on the basis of the force field; on the contrary, only one band shows relative enhancement in the spectra. The involvement of the diketo and dienol forms in the SERS is also discarded given that the theoretical spectra predict the enhancement of bands at 1745 (and 1776) cm^{-1} and 1628 cm⁻¹, respectively, which disagrees with the experimental behaviour. Therefore, only the spectrum of the N1-deprotonated anion agrees with it.

The CT resonant condition is fulfilled in the SERS recorded at 0.0 and -0.25 V (Fig. 3c and d), while the spectrum at -0.5 V seems to be out of resonance given that the band at 1671 cm⁻¹ does not show selective enhancement. The SERS on silver sols show a similar behaviour and the band at 1673 cm⁻¹ becomes much weaker in the spectrum at pH = 12 than at pH = 7; it is possible that the ions of the solution modify the electric potential of the interphase in a similar way



Fig. 4 Calculated CT-RR spectra of different species of 5-FU. (a) Neutral molecule (dienol form); (b) N1 anion; (c) N3 anion; (d) neutral molecule (diketo form). The SERS spectrum on silver sol at (e) pH = 7 is also shown.

to what the imposed potential in the experiences on the electrode.

4. Conclusions

Calculations of vibrational wavenumbers of neutral and N1 and N3 deprotonated ions of 5-FU have been carried out at the B3LYP/6-31G* level of theory. On the basis of the respective force fields it has been possible to identify the two deprotonated tautomers in the Raman spectra of the aqueous solutions recorded at alkaline pH, being the two marker bands recorded at 1350 and 1330 cm⁻¹ for the N1 and N3 anions, respectively. SERS spectra of 5-FU have been recorded on silver sol and electrode. Theoretical intensities have been calculated according to a resonant CT mechanism for each chemical species in order to detect the participation of this enhancement mechanism and to identify which molecular form gives rise to the spectra. The calculated intensities for this SERS-CT process allows us to deduce that the N1 deprotonated anion is responsible for the SERS given that it accounts for the selective enhancement of the band recorded at ca. 1680 cm⁻¹. Since all the strong SERS bands correspond to in-plane modes and no out-of-plane bands are recorded it is expected that 5-FU lies almost perpendicular to the metallic surface.

Acknowledgements

This work was supported by the Spanish MEC (projects NAN2004-09312C03-01, FQM1895 and CTQ2006-02330) and the Portuguese Foundation for Science and Technology (FCT)—Laboratório Associado CICECO. MS and CR also acknowledge FCT and MEC for their PhD grants SFRH/BD/23400/2005 and BES-2007-17250, respectively.

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