The effect of the pH on the interaction of L-arginine with colloidal silver nanoparticles. A Raman and SERS study

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Raman and surface enhanced Raman scattering (SERS) spectroscopies were used to study the pH effect (7 to 9) on the interaction of arginine (Arg) with colloidal Ag nanoparticles (AgNps). A new methodology was implemented in order to obtain reproducible SERS spectra in solution. The dependence of the Arg concentration on the stability of the AgNps is discussed. A pH increasing of the colloidal solution to the limits of the Arg pKa_2 value induces a preferential and stable Arg–metal interaction. ξ potential measurements of the Arg–AgNps system at different pH conditions studied provide information about the Arg–AgNps interaction; the pH increasing favors the interaction. SERS spectra at pH 7 indicate that the molecule interacts with the Ag surface only through the guanidinium fragment. By increasing the pH to 9, the molecule adopts a new conformation on the surface; the metal–analyte interaction is verified through the guanidinium, carboxylate and the aliphatic moieties. In addition, theoretical calculations performed by using the extended Hückel method for a model of Arg interacting with an Ag surface support the observed SERS results. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: L-arginine; Raman spectroscopy; zeta potential (ξ); surface enhanced Raman scattering (SERS); extended Hückel theory (EHT)

Introduction

Raman and surface enhanced Raman scattering (SERS) spectroscopies are techniques widely used to study biological materials such as amino acids, peptides and proteins.[1–4] SERS spectroscopy is an analytical tool allowing Raman intensity enhancements of several orders of magnitude[5]; making it possible to characterize biological molecular systems in solution at very low concentrations (less than 10^{-4} M), deposited on nanostructured metal surfaces.

Several methodologies to obtain SERS activity in aqueous solution of bioanalytes have been developed.[6,7] Some of them allow to obtain spectral reproducibility and analyte control when working with dry samples.[8,9] A SERS spectral reproducibility in aqueous solution is not simple, and it depends greatly on the structural characteristics, the net charge and physicochemical properties such as hydrophilicity[10] of the analyte. Arginine (Arg) and lysine (Lys) have net positive charge at pH near 7.0; both amino acids have basic properties and hydrophilic characteristics. The hydropathy index of an amino acid represents its hydrophobic or hydrophilic properties. Kyte et al.[11] proposed that the most hydrophobic amino acids are isoleucine (4.5) and valine (4.2), while those displaying the highest hydrophilicity are Arg (−4.5) and Lys (−3.9). This concept is very important in the study of the structure of proteins. Hydrophilic amino acids are commonly oriented towards the protein surface.

DiCostanzo et al.[12] described the implications that the Arg–ion metal interactions have on the structure and function of proteins. Vijayakumar et al.[13] registered the Raman and infrared (IR) spectra of Arg–nitrate hemi-hydrate, performing a complete bands assignment supported by HF/6-31G(d) calculations. Petrosian and Sukiasyan[14] studied crystals of Arg·HNO₃·0.5H₂O

and Arg·2HNO₃ by attenuated total reflectance (ATR) and Raman; a complete bands assignment for both crystals was proposed. A temperature-dependent Raman study of Arg hydrochloride monohydrate single crystal was performed by Lima et al.[15] A phase transition was identified, and several spectral changes with the temperature were associated to hydrogen bonding involving the $t(NH_2)$ and $r(NH_3^+)$ deformation modes. Raman bands of Arg were published by DeGelder et al.[16] in a large Raman study of biologic materials. Wolpert and Hellwig[17] published the IR spectrum of several amino acids in solution, including Arg. The band assignment is proposed for Arg at controlled pH of 6.7. Kumar et al.[18] recorded and analyzed the IR and Raman spectra for Arg. These authors also recorded the UV–visible spectrum for aqueous solutions of Arg at different pH values.

Hernández et al.[19] reported the Raman scattering, FT-IR and ATR spectra of aqueous solutions (in H_2O and D_2O) of L-Lys and L-Arg. The authors performed a complete bands assignment supported by theoretical calculations performed with Density Functional Theory at the B3LYP/6-31G* level. Zhu et al.[20] recorded the Raman spectra in the solid and aqueous solutions for 18 amino acids, including Arg; pH conditions are not reported. The pH control should be critical in the study of complex molecular systems as peptides and proteins deposited on metal nanoparticles.

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In previous works with peptides and proteins, we have evidenced the importance of the Lys and Arg residues on the interaction with metal surfaces.[4,8,21,22] In particular, we have demonstrated the inductive effect of the guanidinium moiety on the orientation and interaction of complex biological systems with Ag nanoparticles (AgNps). In the present work, we present the vibrational characterization of the Arg–Ag metal surface interaction at pH 7.0, 8.0 and 9.0 in aqueous solution by using the Raman and SERS techniques, along with UV–visible and zeta potential (ξ) measurements. To our knowledge, vibrational studies concerning the pH effect on the Arg–AgNps interaction have not been published. UV–vis and ξ potential data are proposed to determine the stability of the interacting Arg–AgNps system. In addition, in order to interpret the SERS results, we perform theoretical calculations by using the extended Hückel theory method for a model of Arg interacting with an Ag surface. Thus, we expect to give some structural vibrational insights of biological molecular systems interacting with metal surfaces in aqueous solution at controlled pH.

Experimental

Sample

L-Arg hydrochloride solid was purchased from Merck and used as received. Stock solutions of Arg in nanopure water (conductivity 18.2 MΩ) were prepared to a final concentration of 10^{-2} M.

Preparation of silver nanoparticles

AgNps were prepared by chemical reduction of silver nitrate with hydroxylamine hydrochloride.[23] The resulting colloid displays a final pH value close to 7. The aqueous solutions utilized for the AgNps formation were prepared by using nanopure water. The colloid displays a milky grey color.

Sample preparation for the Raman, SERS, UV–visible and ξ measurements

Raman measurements were performed in Arg crystals deposited on a quartz slide. Arg aqueous solution was prepared adding 0.871 g of solid Arg to 5.0 ml of nanopure water; the solution was stirred during 6 h using a Vortex. Then, the solution stands for 24 h at room temperature. The final solution concentration is close to 1 M. 6 μl were used for the Raman measurements. SERS measurements were carried out for three identical samples; they were prepared by mixing 0.5 ml of Arg 10^{-2} M with 3.0 ml of the colloidal solution. 7.5 ml of nanopure water was added in order to obtain a final analyte concentration 4.5×10^{-4} M. This system settles down for 30 min. The resulting pH value was close to 8.0. The final pH was adjusted to 7.0, 8.0 and 9.0 by adding drops of aqueous solutions of NaOH or HCl 0.05 M. The best reproducible SERS spectra were obtained immediately after the desired pH value was obtained. The pH value changes from 45 min. Raman and SERS measurements were performed at room temperature. UV–visible spectra and ξ potential measurements were performed for the samples without any additional preparation.

Instrumentation

The Raman and SERS spectra of Arg were scanned with a Renishaw microRaman system (RM1000) equipped with the 514,

633 and 785 nm excitation laser lines, a Leica microscope and an electrically cooled CCD camera. The signal was calibrated by using the 520 cm^{-1} line of a Si wafer and a 50× objective. The laser power on the system was less than 2 mW. The resolution was set to 4 cm^{-1}, and 1–6 scans of 10 s were averaged. Spectra were recorded in the 200–1800 cm^{-1} region. Sample was photo stable when probed with laser lines at 633 and 785 nm. The best spectral data were obtained by using the 785 nm laser line for the SERS measurements and the 633 nm laser line for the Raman measurements. The UV–vis measurements were performed by using a diode array spectrophotometer Hewlett Packard 8452 A. A Zeta sizer 3000 Malvern Instrument was used for the ξ potential measurements.

Experimental setup for SERS measurements in colloidal aqueous solution

A transparent plastic tip for micropipette with capacity up to 10 μl was used as a sample holder for the SERS measurements (see Fig. 1). This system allowed scanning the SERS spectra in colloidal aqueous solution directly within the solution. This procedure guarantees spectral reproducibility working with very small volume of sample (6 μl). 20× and 50× microscope objectives were used.

Molecular model and calculations

A simplified molecular model for the Arg–metal surface interaction was built as in our previous study.[9] A face centered-cubic structure with d(Ag-Ag) = 4.08 Å was built and trimmed to get a planar bilayer composed of 324 Ag atoms. Arg displays a neutral amine group. Molecular mechanics at the OPLS parameterization level was used to optimize the Arg–Ag geometry, keeping constant the Ag layer geometry. Arg was placed at different distances and orientations from the center of the Ag layer. EHT was used to calculate the wave function of Arg, the Ag layer and the Arg–Ag system. EHT calculations produce adequate qualitative or semi quantitative descriptions of the molecular orbital and electronic properties.[24,25] The Hyperchem program was used.[26] The present methodology was successfully used in our previous SERS works in peptides,[4,21,22] nanotubes,[27] tryptophan,[28] Lys,[7] humic acids[29] and proline[2] interacting with Ag surfaces.
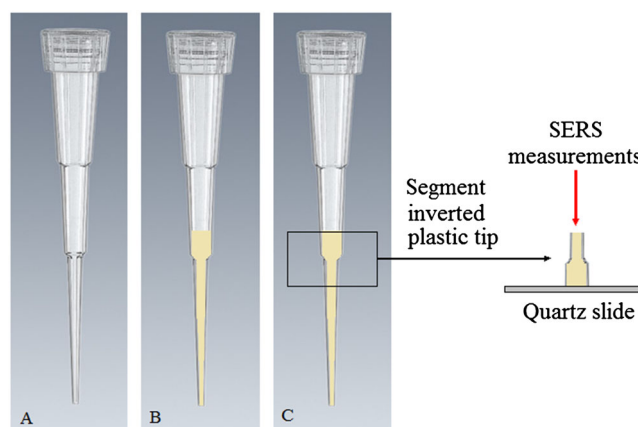

Figure 1. Schematic sample holder for the SERS measurements.

Results and discussion

Physicochemical properties of L-Arg

Different structures for Arg predominate at specific pH ranges as displayed in Fig. 2. Protonation of the carboxyl groups occurs at a low pH; the positively charged guanidinium group displays a protonated form. The carboxyl and carboxylate forms are in equilibrium at pH = pK_{a1}. Likewise, the α-amino group reaches an equilibrium between the protonated (NH_3^+) and the non protonated (NH_2) forms at pH = pK_{a2} (see pK_a values in table 1). Upon reaching this pH, the guanidinium group keeps the +1 net charge, while the positive charge on the α-amino group decreases. Thus, the guanidinium group is expected to interact with the silver nanoparticles displaying a preferential orientation on the metal surface; the chloride ions remaining from the synthesis of the AgNps impose a negative environment on the metal surface. The ξ potential value of the AgNps is negative in a large pH range.[30] Under these conditions, about 50% of the C species have a non-protonated α-amino group (see Fig. 2). Thus, the positive charge of the amino acid is placed on the guanidinium group. Therefore, it is expected that the Arg–AgNp interaction takes place mainly through the guanidinium group at pH near 9.0.

Stability of the colloidal silver solution

UV–visible measurements

A colloidal stability is inferred from the absorption spectrum at pH values of 7, 8 and 9 colloidal solutions. The colloidal stability also depends on the analyte concentration. Figure 3 displays the absorption spectra of the pure silver colloid (red) and the colloidal solutions containing Arg in final concentration 4.5×10^{-4} and 5×10^{-3} M. The absorption spectrum of pure silver colloid displaying a maximum at 414 nm, shifts in the Arg 4.5×10^{-4} M solutions to 422 nm at pH 7 and 8, and shifts to 424 nm at pH 9.0. (See spectra a, b and c in Fig. 3). At concentrations higher than 10^{-3} M of Arg the metallic colloid is destabilized. The absorption spectrum of these systems displays broad bands and a maximum shifting to 406 nm. (See spectra d, e and f in Fig. 3). The best concentration conditions are found at concentrations 4.5×10^{-4} M. From the present absorption spectra of the AgNps–Arg system at different pH conditions, showing rather identical spectral profiles and identical to the pure silver colloidal solution, we inferred that the nanoparticles remain with similar structural characteristics (see the zeta
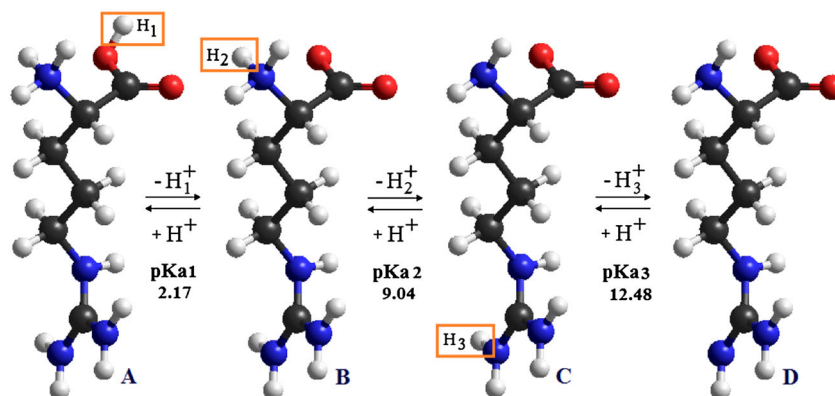
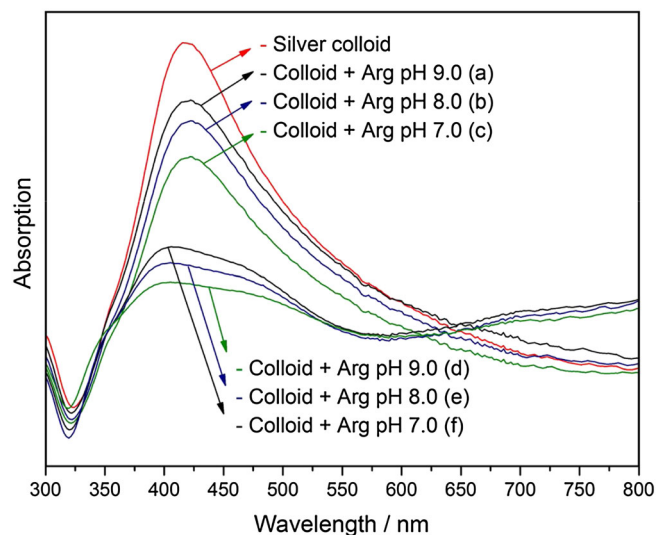| Table 1. Some physicochemical properties of arginine		
pK_a values for Arg	Approximate net charge	Isoelectric point
$pK_{a1} = 2.17$	>1.0	11.04
$pK_{a2} = 9.04$	~1.0	Hydropathy index
$pK_{a3} = 12.48$	<0.0	−4.5

Figure 3. Absorption spectra of the silver colloid arginine in colloidal solution 4.5×10^{-4} M at pH 9.0, 8.0 and 7.0 (a,b,c) and in colloid solution 5×10^{-3} M at pH 9.0, 8.0 and 7.0 (d,e,f).

potential results) and that the colloidal systems maintain the stability conditions in the pH 7–9 range.

Zeta potential

ξ potential measurements for the silver colloid and colloidal aqueous solutions containing Arg in concentration $4,5 \times 10^{-4}$ M at pH 7, 8 and 9 were carried out. The Ag colloid displays ξ potential values of −43.0, −46.6 and −51.3 mV at pH 7, 8 and 9, respectively. This result agrees with that proposed by Alvarez-Puebla *et al.*[30]; ξ potential becomes more negative by pH increasing. An opposite trend is verified for the ξ potential values of the colloid–Arg solutions: ξ potential increases from −43.5 to −35.2 mV by increasing the pH from 7 to 9, see Fig. 4. Thus, the negative charge decreasing on the surface of silver
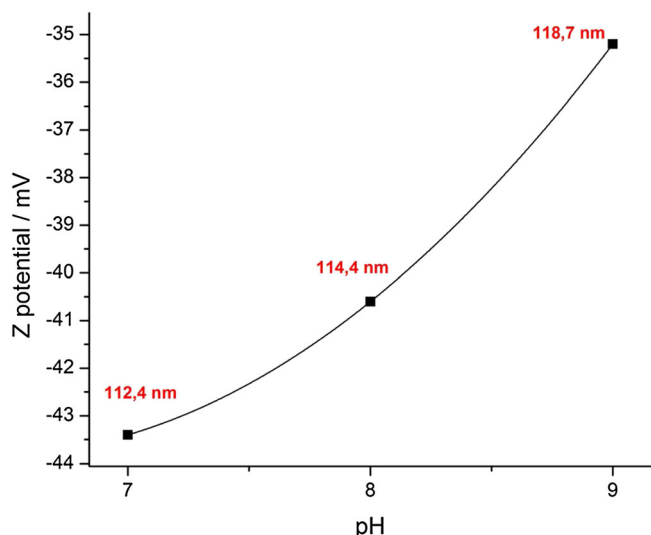

Figure 2. Arginine structure at different pH values.

Figure 4. Variation of ξ potential with pH, indicating the average diameters of silver nanoparticles at each pH.

Figure 5. Raman spectra of arginine (a) aqueous solution and (b) solid. Laser line 633 nm.

nanoparticles is due to the Arg–AgNp interaction. The total positive charge on the Arg molecules decreases by increasing the pH solution; the amino acidic molecular fragment polarity decreases, and the whole molecule becomes less hydrophilic. Thus, the analyte addition probably reduces the negative charge
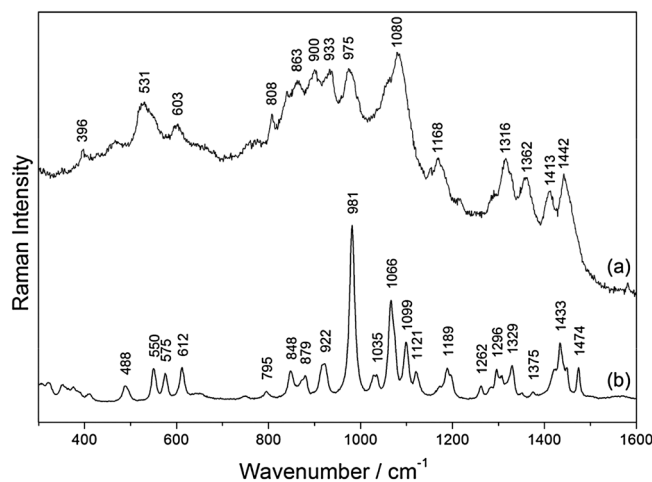
on the nanoparticles, and a charge redistribution on the colloidal system should occur. The positively charged guanidinium moiety observes at the higher pH a colloidal system which facilitates the analyte–surface interaction. Moreover, the analyte becomes less hydrophilic which is an additional argument to approach the metal surface. These results lead us to suggest that Arg at pH 9 approaches the surface at shorter distances than at lower pH;

Table 2. Raman of solid, Raman aqueous solution and SERS at pH 7.0, 8.0 and 9.0 wavenumbers (cm^{-1}) with relative intensity[a] of arginine and the most probable bands assignment

Raman Arg solid sample	Raman Arg aqueous solution	SERS pH 7.0	SERS pH 8.0	SERS pH 9.0	Most probable assignment
				1653 w	δ NH/ν C = N
1474 m				1573 w	δ NH
					δ CH$_2$
1433 m	1442 s	1447 wm	1447 w	1445 ms	δ NH (guanid.)
	1413 m	1410 vw	1402 w	1400 m	ν COO$^-$
1375 w	1362 s		1351 vw	1331 ms	δ CH$_2$
1329 m	1316 s		1322 vw		
1296 m		1273 wm	1297 vw	1296 w	δ CH$_2$
					δ CH$_2$
1262 w				1229 w	δ CH$_2$
1189 m	1168 m	1167 vw	1157 vw	1164 w	δ NH
1121 m		1135 vw			r NH$_3^+$
					ρ NH$_2$
1099 m	1080 vs	1083 wm	1088 ms	1086 s	δ NH (guanid.)
1066 s					
1035 w			1030 vw	1036 w	ω NH$_3^+$
981 vs	975 s	980 vw	976 vw	928 s	ρ CH$_2$
922 m	933 m		934 w		ν C–COO$^-$
	900 m				ν C–N
879 w	863 m	845 w	850 w	850 m	ν C–C
848 w	808 w				ω N–H
					ν C–C
795 w		772 vw	766 w	750 w	ρ CH$_2$
612 m	603 wm	661 w	666 m	663 s	δ COO$^-$
575 m	531 m	574 w	572 m	561 w	δ COO$^-$
550 m					δ COO$^-$
					τ NH$_3^+$, τ NH$_2$

[a]*Relative intensity: vs, very strong; s, strong; m, medium; ms, medium–strong w, weak; vw, very weak. (guanid.: guanidinium)*

the orientation of its guanidinium group takes place to reach an optimal interaction, which might be achieved through the recognition of the whole molecule of high electric field density regions on the metal surface. Under the above conditions, the carboxylate moiety also approaches the metal surface.

Raman and SERS spectra

Raman

Raman spectra of Arg in solid state and in aqueous solution are shown in Fig. 5. The assignment of the Raman bands in the solid was already discussed.[9] In this work, we scan the Raman spectrum of Arg in aqueous solution. The Raman bands assignment of Arg in aqueous solution was performed following Hernández *et al.*,[19] (see Table 2).

SERS

SERS spectral reproducibility was achieved by using the proposed methodology. The band assignment is shown in Table 2. The spectrum recorded at pH 7.0 shows weak bands. A high SERS spectral definition is obtained by increasing pH. Two bands ascribed to guanidinium NH deformations at $1086\,cm^{-1}$ and $1445\,cm^{-1}$ increase their relative intensity by increasing pH (See Fig. 6). This is consistent with an interaction of Arg with AgNps through the positively charged guanidinium group at pH 9.0. No spectral modifications by pH effect were observed for the α-amine group (NH_3^+) bands. Bands corresponding to the carboxylate group at 663, 928 and $1400\,cm^{-1}$ increase their intensity by increasing pH. The interaction with the silver surface of the carboxylate group involves a displacement of the chloride anions on the AgNps surface. The bands observed at 850, 1229, 1296 and $1331\,cm^{-1}$ in the SERS spectrum at pH 9.0 correspond to vibrational modes involving the Arg aliphatic fragment. The spectral behavior by increasing pH of the aliphatic bands is consistent with an interaction of the aliphatic fragment with the AgNps. The whole spectral modifications observed by increasing pH effect are interpreted in terms that the Arg molecule interacts with the metal surface through the guanidinium, carboxylate and the aliphatic moieties. Our previous theoretical calculations at pH 7.0 indicated that the Arg molecule interacts with the Ag surface through the guanidinium group, where the Arg orientation on the surface is directed by the guanidinium group, the whole molecule is oriented rather perpendicular to the surface.[9] According to the present results the orientation on the surface of the Arg molecule evolves from a nearly perpendicular to a plane parallel configuration. The present calculations for the amino acid performed at pH 9.0 support the experimental issues.

Theoretical aspects

In the silver metallic bilayer, the valence and conduction bands overlap,[31] thereby indicating that this model represents well a metal surface. The π character of the HOMO and LUMO are located over almost all the Ag atoms with the exception of the center of the layer. Regarding Arg, the highest occupied MO has π components located on the O atoms and on the guanidinium group. The main Arg–Ag distances are in the 3.0–3.3 Å range for the O–Ag interaction and in the 2.9–3.0 Å range for the guanidinium (N atoms)–Ag interaction. The final geometry predicted by calculations is shown in Fig. 7. Calculations performed with the chemical structure of Arg at pH 9.0, support the experimental SERS results; at pH 9.0 Arg interacts
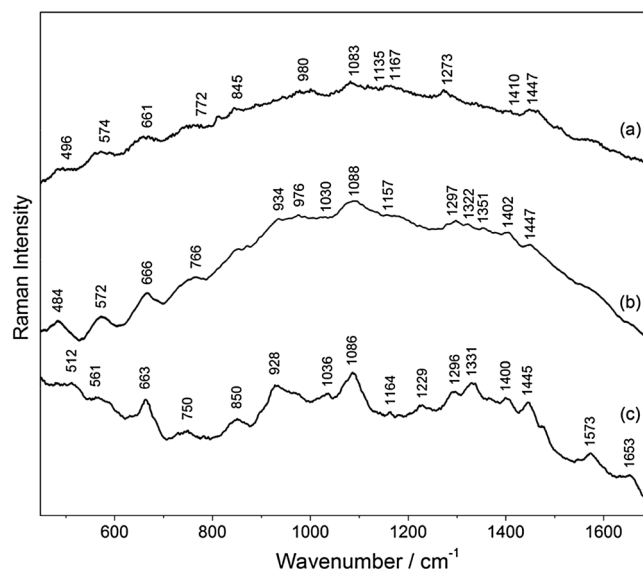

Figure 6. SERS spectra of arginine in colloidal solution at pH 7.0 (a), 8.0 (b) y 9.0 (c). Laser line 785 nm.
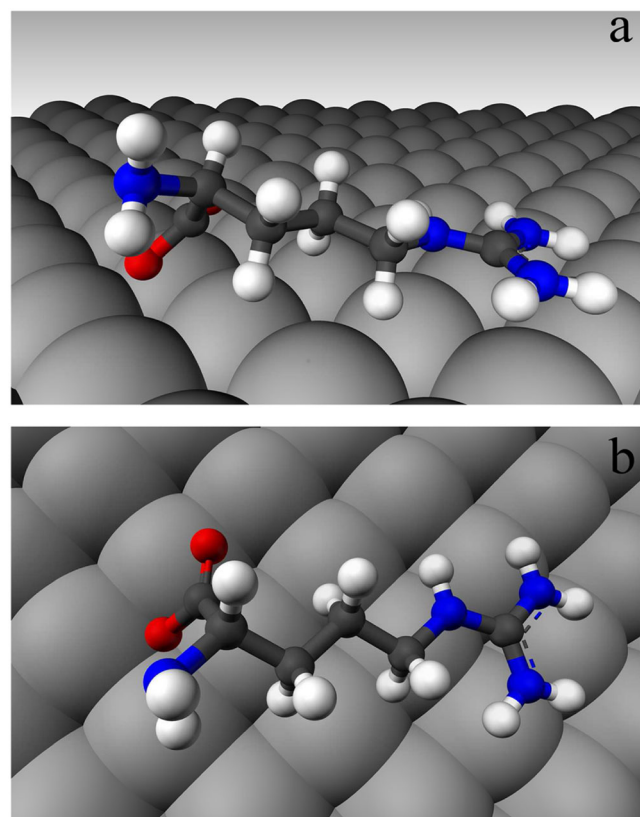

Figure 7. Predicted molecular model for the Arg–Ag surface interaction. (a) side view and (b) top view.

with the metal surface through the guanidinium, carboxylate and the aliphatic fragments. In fact, and following the SERS selection rules[32] indicating that modes having their Raman polarizability *z*-component perpendicular to the surface are likely to become more enhanced than the parallel ones, some particular vibrations of the guanidinium group for instance,

should be enhanced the most; this is the case for the guanidinium vibrations at 1086 and 1445 cm^{-1}, see Fig. 6.

Conclusions

A new methodology is proposed to obtain Raman and SERS spectral reproducibility for samples in solution. On this basis, it is concluded that the Arg–AgNps interaction in aqueous solution is highly dependent on the pH. The Ag colloidal solution kept its physicochemical electrical properties for the Arg concentration 4.5×10^{-4} M and pH 7–9 range. A pH increasing of the colloidal solution to the limits of the Arg pKa$_2$ value induces a preferential and stable Arg–metal interaction. The UV–visible and ξ potential measurements allowed us to evaluate the stability of the system Arg–AgNps at the different pH conditions studied. The ξ potential measurements also provide information about the interaction Arg–AgNps; the pH increasing favors the analyte–metal surface interaction. At pH 7, the molecule interacts weakly with the AgNps only through the guanidinium fragment. By increasing the pH to 9, the molecule trends to adopt a new conformation on the surface; the metal–analyte interaction is verified through the guanidinium, carboxylate and the aliphatic moieties. Theoretical calculations performed by using extended Hückel theory method for a structural model of the Arg–Ag layer interaction support the SERS results.

Acknowledgements

References

[1] R. Weidong, J. Wei, X. Xiangxin, C. Yang, C. Lei, Z. Tieli, N. Li, L. Xiao, Z. Junhu, Z. Bing, *J. Raman Spectrosc.* **2011**, *42*, 1492.

[2] J. J. Cárcamo, A. E. Aliaga, E. Clavijo, C. Garrido, J. S. Gómez-Jeria, M. M. Campos-Vallette, *J. Raman Spectrosc.* **2012**, *43*, 750.

[3] S. Stewart, P.M. Fredericks, *Spectrochim. Acta A* **1999**, *55*, 1641.

[4] A. E. Aliaga, T. Aguayo, C. Garrido, E. Clavijo, E. Hevia, J. S. Gómez-Jeria, P. Leyton, M. M. Campos-Vallette, S. Sánchez-Cortes, *Biopolymers* **2011**, *95*, 135.

[5] R. Aroca, Surface-enhanced Vibrational Spectroscopy, John Wiley & Sons, Chichester, **2006**.

[6] G. Das, F. Mecarini, F. Gentile, F. De Angelis, M. Kumar, P. Candeloro, C. Liberale, G. Cuda, E. Di Fabrizio, *Biosens. Bioelectron.* **2009**, *24*, 1693.

[7] A. E. Aliaga, I. Osorio-Román, C. Garrido, P. Leyton, J. Cárcamo, E. Clavijo, J.S. Gómez-Jeria, G. Díaz-Fleming, M.M. Campos-Vallette, *Vib. Spectrosc.* **2009**, *50*, 131.

[8] C. Garrido, A. E. Aliaga, J. S. Gómez-Jeria, R. E. Clavijo, M. M. CamposVallette, S. Sánchez-Cortes, *J. Raman Spectrosc.* **2010**, *41*, 1149.

[9] A.E. Aliaga, C. Garrido, J. S. Gómez-Jeria, P. Leyton, G. Díaz-Fleming, J. Cárcamo, E. Clavijo, M.M. Campos-Vallette, S. Sanchez Cortes, *Spectrochim. Acta A* **2010**, *76*, 458.

[10] T. P. Hoop, K. R. Woods, *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 3824.

[11] J. Kyte, R. F. Doolittle, *J. Mol. Biol.* **1982**, *157*, 105.

[12] L. DiCostanzo, L.V. Flores, D.W. Christianson, *Protein. Struct. Funct. Bioinf.* **2006**, *65*, 637.

[13] T. Vijayakumar, I. H. Joe, C. P. Reghunadhan-Nair, V.S. Jayakumar, *J. Raman Spectrosc.* **2009**, *40*, 18.

[14] A. M. Petrosian, R. P. Sukiasyan, *J. Mol. Struct.* **2008**, *874*, 51.

[15] R. J. C. Lima, P. T. C. Freire, J. M. Sasaki, F. E. A. Melo, J. M. Filho, *J. Raman Spectrosc.* **2002**, *33*, 625.

[16] J. De Gelder, K. De Gussem, P. Vandenabeele, L. Moens, *J. Raman Spectrosc.* **2007**, *38*, 1133.

[17] M. Wolpert, P. Hellwig, *Spectrochim. Acta A* **2006**, *64*, 987.

[18] S. Kumar, S. B. Rai, *Indian J. Pure Appl. Phys.* **2010**, *48*, 251.

[19] B. Hernández, F. Pflüger, N. Derbel, J. De Coninck, M. Ghomi, *J. Phys. Chem. B* **2010**, *114*, 1077.

[20] Z. Guangyong, Z. Xian, F. Qi, W. Xueliang, *Spectrochim. Acta A* **2011**, *78*, 1187.

[21] A. E. Aliaga, H. Ahumada, K. Sepúlveda, J. S. Gómez-Jeria, C. Garrido, B. E. Weiss-López, M. M. Campos-Vallette, *J. Phys. Chem. C* **2011**, *115*, 3982.

[22] C. Garrido, A. E. Aliaga, J. S. Gómez-Jeria, J. J. Cárcamo, E. Clavijo, M. M. Campos-Vallette, *Vib. Spectrosc.* **2012**, *61*, 94.

[23] N. Leopold, B. Lendl, *J. Phys. Chem. B* **2003**, *107*, 5723.

[24] J. S. Gómez-Jeria, *J. Comput. Theor. Nanosci.* **2009**, *6*, 1361.

[25] W. Koch, B. Frey, J. F. Sanchez, T. Scior, *Z. Naturforsch.* **2003**, *58*, 756.

[26] Hypercube Inc. 1115 NW 4th Street, Gainesville, Fl. 32601, USA.

[27] P. Leyton, J. S. Gómez-Jeria, S. Sánchez-Cortes, C. Domingo, M. M. Campos-Vallette, *J. Phys. Chem. B* **2006**, *110*, 6470.

[28] A. E. Aliaga, P. Leyton, I. Osorio-Román, C. Garrido, J. Cárcamo, C. Caniulef, F. Célis, G. Díaz-Fleming, E. Clavijo, J. S. Gómez-Jeria, M. M. Campos-Vallette, *J. Raman Spectrosc.* **2009**, *40*, 164.

[29] P. Leyton, I. Córdova, P. A. Lizama-Vergara, J. S. Gómez-Jeria, A. E. Aliaga, M. M. Campos-Vallette, E. Clavijo, J. V. García-Ramos, S. Sánchez-Cortes, *Vib. Spectrosc.* **2008**, *46*, 77.

[30] R. A. Álvarez-Puebla, E. Arceo, P. J. G. Goulet, J. J. Garrido, R. F. Aroca, *J. Phys. Chem. B* **2005**, *109*, 3787.

[31] J. S. Gómez-Jeria, *J. Chil. Chem. Soc.* **2006**, *51*, 1061.

[32] M. Moskovits, *Rev. Mod. Phys.* **1985**, *57*, 783.