Study of Interaction Between Aspartic Acid and Silver by Surface-Enhanced Raman Scattering on H₂O and D₂O Sols

J. F. ARENAS, J. L. CASTRO, J. C. OTERO, J. I. MARCOS
Department of Physical Chemistry, University of Málaga, E-29071 Málaga, Spain

Received 7 December 2000; revised 26 February 2001; accepted 12 April 2001

ABSTRACT: Three different surface-enhanced Raman scattering (SERS) spectra are recorded for aspartic acid on H₂O silver sols under different concentrations and pH values. The analysis of the results shows that it interacts with the metal surface in its dianionic form in two different ways, depending on the pH and concentration. Moreover, in some cases the fumarate anion is detected, which results from the chemical surface transformation of the aspartate. The N-deuterated aspartic acid adsorbed on the D₂O silver sols gives rise to only one SERS spectrum as a consequence of the interaction of amino and carboxylate functional groups of the dianion with the metal, independent of the concentration and pD. © 2001 John Wiley & Sons, Inc. Biopolymers (Biospectroscopy) 62: 241–248, 2001

Keywords: surface-enhanced Raman scattering; aspartic acid; Raman spectrum; vibrational spectrum

INTRODUCTION

The vibrational study of amino acids adsorbed on metal surfaces is important because it can yield insight into the metal–biopolymer interactions. These molecular systems are interesting given that the presence of amino and carboxylate functional groups can in principle be responsible for the interaction with the metal surface. In this respect, surface-enhanced Raman scattering (SERS) has proved to be a powerful technique to derive valuable conclusions about this kind of intermolecular information. Among the amino acids studied by SERS, glycine, α-alanine, 6-aminocaproic acid, valine and leucine, cystine, cysteine and methionine, α-aminobutyric acid, and γ-aminobutyric acid (GABA) were found to interact through both functional groups. A few of them such as cystine, β-alanine, and β-aminobutyric acid interacted in different ways, depending on the pH. For instance, cysteine interacted at a pH of <7 through either the sulfur atom or the carboxylate group or both, but at a pH of >7 it also interacted through the amino group.

Aspartic acid, which has two carboxylic groups, is a more complex system because it can be considered as both an α- and β-amino derivative. Given that it contains two carboxylic groups, it can be present in aqueous solution as one of the four following ionic species:

\[
\begin{align*}
\text{HOOC–CH}_2\text{–CH(NH}_3^+\text{)–COOH} \\
\text{HOOC–CH}_2\text{–CH(NH}_3^+\text{)–COO}^- \\
\text{\text{OOC–CH}_2\text{–CH(NH}_3^+\text{)–COO}^-} \\
\text{\text{OOC–CH}_2\text{–CH(NH}_2\text{)–COO}^-}
\end{align*}
\]

Correspondence to: J. C. Otero (jc.otero@uma.es).
Contract grant sponsor: DGI; contract grant number: BQU2000-1253.
In addition, species V could be found adsorbed on the metal surface, although it cannot be considered as macroscopically stable in solution, and therefore its Raman spectrum cannot be recorded:

\[
\text{HOOC—CH}_2—\text{CH(NH}_2\text{)—COO}^–
\]

Taking into account the successive pK values corresponding to the formation of species I–IV (pK\(_1\) = 2.10, pK\(_2\) = 3.86, and pK\(_3\) = 9.82, respectively) and a pH of 2.98, it is possible to deduce that the majority species in a 1M aqueous solutions at pH 1, 3, 7, and 12 are I, II, III, and IV, respectively.

Chumanov et al.\(^2\) carried out a general study of the SERS of amino acids and classified them into three groups on the basis of the molecular mechanism of their interaction with the surface: the aspartic and glutamic acids are included in the group where interaction is through the carboxylate and the amino groups. In a preliminary work published by our group, the SERS of aspartic acid was correlated with the respective SERS spectra of aliphatic dicarboxylic acids\(^6\) and with the Raman spectra in aqueous solution at different pH values in order to perform a complete assignment of the SERS bands with the help of a computed force field.\(^7\) These previous studies are completed in this article where three different SERS spectra from aspartic acid on H\(_2\)O silver sols are reported, which depended on the concentration of the adsorbate and the pH. At low concentrations in a basic or neutral medium the results seem to point out that the adsorbed species is dianion IV through the interaction of amino and carboxylate groups. When the concentration is increased to 10\(^{-2}\)–10\(^{-3}\) M, the involved species at pH 7 seems to be adsorbed only through the side carboxylate group. On the other hand, we recorded a SERS spectrum at 10\(^{-3}\) M and pH 7 that can be explained as a double adsorption of aspartate and fumarate anions originating from a chemical transformation yielding fumaric acid.

We also studied the SERS spectra of aspartic acid adsorbed on D\(_2\)O silver sols at different concentrations of adsorbate and pH values. This kind of work on D\(_2\)O is very scarce and only the SERS of a complex amino acid, p-amino benzoic acid,\(^8\) is found in the literature. Its usefulness is to assign modes involving the amino group and to confirm the most relevant conclusions derived from the SERS recorded on H\(_2\)O sols. Previous to this work, we studied GABA\(^5\) and glycine\(^9\) in a deuterated colloidal medium. The former interacts at concentrations over 10\(^{-3}\) M through both functional groups, and its behavior is the same in a nondeuterated medium; at even lower concentrations identical results are obtained but some spectra also show the so-called anomalous SERS spectrum\(^10\) typical of small organic molecules with a carboxylic group. Concerning glycine in D\(_2\)O, the adsorbed species is the glycinate-N-d\(_2\) anion and the interaction is through carboxylate and amino groups.

**EXPERIMENTAL**

Colloidal silver solutions were prepared in deionized and triply distilled water according to the method described by Creighton et al.\(^11\) which was basically reducing a solution of 10\(^{-3}\) M AgNO\(_3\) with an excess of NaBH\(_4\). One volume of 10\(^{-3}\) M AgNO\(_3\) was added dropwise with strong stirring to 3 vol of 2 \(\times\) 10\(^{-3}\) M NaBH\(_4\), which was previously cooled to a temperature between 0 and 5°C. After stirring the mixture for some time to homogenize it, it was allowed to rest at room temperature for approximately 90 min. When a dark color appeared in the heart of the solution, vigorous stirring was necessary to stabilize the colloidal solution. This was a transparent, yellow solution with a maximum in its absorption spectrum at 390 nm. Adsorbate was then added to the colloid as an aqueous solution to obtain the desired concentration, and a change in the color of the system from the initial yellow to a final greenish-blue was observed that was due to aggregation.

The colloidal solutions in deuterated water were obtained by a similar method using D\(_2\)O as the solvent. N-Deuterated amino acids were prepared by dissolving the nondeuterated reagents in D\(_2\)O and recrystallizing them thereafter. A further dissolution of the deuterated amino acids in NaOD medium resulted in the respective N-deuterated anions.

The Raman spectra were recorded with a Jobin–Yvon U-1000 double monochromator spectrometer fitted with a cooled Hamamatsu R943-02 photomultiplier and using the 514.5-nm exciting line from a Spectra Physics 2020 Ar\(^+\) laser. A constant slit width was used that allowed a spectral resolution of 4 cm\(^{-1}\); the laser power reaching the sample was always 60 mW. In the liquid samples a quartz cell with a 1-cm pathlength was used, and a glass capillary was used for the microcrystalline solids. The measurement
of the band frequencies was done with the help of the same laser plasma lines as the frequency standards, whereby a precision of $\pm 2\text{ cm}^{-1}$ was obtained under the operating conditions employed.

RESULTS AND DISCUSSION

The SERS records were analyzed according to the standard methodology. The frequency shifts of the SERS bands were studied first to establish whether the molecule was adsorbed or not and which part of it was linked to the metal. Then the so-called propensity rules of the electromagnetic enhancement mechanism were applied. According to these, the most enhanced bands must correspond to vibrations involving that part of the molecule closest to the metal surface and with an orientation perpendicular to it.

H$_2$O Silver Sols

Figure 1 shows the Raman spectra of an aqueous solution of 1M aspartic acid at pH 0, 7, and 14. These spectra should correspond to species I, III, and IV, respectively. In the pH close to the isoelectric point (species II) the aspartic was barely soluble, which hindered the recording of the spectra. The Raman spectrum recorded at pH 0 was characterized by the strong intensity of the band at 1734 cm$^{-1}$ that was assigned to the $\nu$(C=O) stretching of the acid group. That band was missing in the SERS spectra of aspartic acid recorded at pH 7 and 12 (Fig. 2). In turn, the symmetric stretching $\nu_s$(OCO) of the carboxylate was recorded but shifted from the original frequency at about 1415 cm$^{-1}$ in the Raman spectrum down to about 1390 cm$^{-1}$ in SERS. All these facts eliminated the participation of species I, II, and V in the SERS spectra and pointed out that aspartic adsorbs through the carboxylate group. Figure 2(a) shows the SERS spectrum of a $5 \times 10^{-3}$ M solution at pH 7, and similar SERS were obtained from solutions with concentrations between $10^{-2}$ and $10^{-3}$ M with some strong bands in the central region. The sharp look of these bands may have been due to a well-formed silver complex. In the Raman spectra recorded at pH 7 and 14, the bands recorded in the 200–1800 cm$^{-1}$ region had quite similar frequencies. The most important difference was the band recorded at 3308 cm$^{-1}$ at pH 14, which was assigned to $\nu$(NH$_2$) of species IV. Concerning the intensities, the Raman recorded at pH 7 showed two strong bands at 853 and 942 cm$^{-1}$ while at pH 14 only the latter band was strong at 947 cm$^{-1}$.

The frequencies in the SERS recorded at pH 7 [Fig. 2(a)] could be correlated with any of the Raman ones shown in Figure 1(b,c), but the band at 942 cm$^{-1}$ is much stronger than the one at 863 cm$^{-1}$ that occurs in the Raman spectrum of species IV at pH 14 [Fig. 1(c)]. This behavior agreed with that observed in the SERS of other molecules with ionizable groups, given that the positive charge of the surface excluded the H$^+$ ions. Under such conditions the local pH in the interphase was higher than the bulk one. Consequently, the SERS spectrum of Figure 2(a) seemed to be originated by the dianionic species IV, although this was not a definitive conclusion. The interacting group must have been a carboxylate in view of the strong relative intensity and the redshift of the band at 1398 cm$^{-1}$, which was
assigned to \( \nu_s(OCO) \). In principle, it was difficult to decide which of the two carboxylate groups was involved in the adsorption. However, it was observed experimentally that \( \alpha \)-amino acids without a side functional group were adsorbed with difficulty while aspartic acid was adsorbed quickly in a wide range of concentrations. For instance, aspartic acid yielded a colloid aggregation much faster than that originated by 2-aminobutyric acid, which also possesses four carbon atoms.14 All of these results seemed to point out that aspartic acid links to the metal surface through its \( \beta \)-carboxylate group. The facts implied that the strongest SERS band in the region between 800 and 1100 cm\(^{-1}\), which was recorded at 942 cm\(^{-1}\), must be assigned to the C\(\equiv\)O stretching of the C\(\equiv\)O\(\equiv\)COO bond. This fundamental was recorded at a similar frequency in the Raman spectrum of the solutions at pH 7 and 14 while it appears at 937 cm\(^{-1}\) in the Raman spectrum of the solid.6,14 These empirical correlations were also in agreement with the results of the theoretical calculations of the vibrational spectrum of aspartic acid.6

The previous analysis of the SERS recorded at pH 7 [Fig. 2(a)] allowed the proposal of the orientation of species IV in a completely trans conformation as shown in Figure 3. The NH\(_2\) group and the H\(_2\) should be far of the metal surface. The methylene group was close enough to the surface, given that vibrations \( \delta(CH_2) \) and \( \nu_s(CH_2) \) were rather strong at 1412 (sh) and 2930 cm\(^{-1}\), respectively.

When the pH was increased to 12 by the addition of aqueous NaOH, the SERS spectrum was significantly changed as shown in Figure 2(b). All the recorded bands became much broader and some were strongly enhanced such as those at 1056 and 3268 cm\(^{-1}\), especially that at 1056 cm\(^{-1}\), which must be assigned to the \( \nu(CN) \) vibration. Others were weakened like those recorded at 861 and 947 cm\(^{-1}\).6 The SERS band at 3268 cm\(^{-1}\) undoubtedly corresponded to \( \nu_s(NH_2) \); it was missing in the Raman at pH 7, which discarded the adsorption of species III, given that no vibrations of the NH\(_3\) functional group were detected in any SERS. The frequency of this SERS band was also redshifted with respect to that recorded at 3308 cm\(^{-1}\) in the Raman spectrum, pointing out that a molecular reorientation took place in such a way that the amino and carboxylate groups interacted simultaneously with the surface to form a chelate. This particular band was also observed in the SERS recorded at pH 7 [Fig.
which could indicate that the chelate was also present at this pH, although to much less of an extent provided that the intensity was much weaker. Adsorption of species IV through both the amino and carboxylate groups gave a pseudocyclic complex with the metal surface that could have originated the band recorded at 330 cm$^{-1}$ in the spectrum of Figure 2(b). A quite similar band was recorded in the SERS spectra of succinic acid, among others.$^{15}$

However, some samples of this adsorbate gave rise to the SERS spectrum shown in Figure 4(a). That spectrum exhibited bands common to the previously analyzed spectrum; but it also showed medium or strong bands at 754, 1280, and 1648 cm$^{-1}$, as well as a very weak band at 3046 cm$^{-1}$, which were not recorded in the spectra of Figure 2.

The band at 3046 cm$^{-1}$ was characteristic of vinylene CH stretching.$^{16,17}$ This supported the hypothesis that the initially adsorbed species underwent a stereospecific Hofmannlike degradation on the metal surface that led to the anionic species $^\text{−}$OOC—CH=CH—COO$^-$. This hypothesis was supported by the previous study by Moskovits and Suh on the SERS spectrum of fumaric acid.$^{18}$ They reported four medium bands at 361, 753, 1274, and 1394 cm$^{-1}$; a strong band at 1646 cm$^{-1}$; and a very weak one at 3051 cm$^{-1}$.$^{18}$ The SERS of a $10^{-3}$ M solution of fumaric acid is shown in Figure 4(b).

This kind of elimination reaction between the $\alpha$ and $\beta$ positions of carboxylic acids yielded a double CC bond that gave rise to a characteristic strong band in the SERS, which was recorded at higher than 1600 cm$^{-1}$. Our group extensively studied that behavior and called these records "anomalous SERS."$^{10}$ In the anomalous spectrum of aspartic acid [Fig. 4(a)] there were the same bands as in the SERS of fumaric acid at very similar frequencies and intensities, although the band at 1396 cm$^{-1}$ was merged into the $\nu$(OCO) of the aspartate anion. According to Moskovits and Suh,$^{18}$ the fumarate dianion is adsorbed parallel to the surface through the $\pi$-ethylenic orbital. Thus, for this reason the $\nu$(C==C) vibration recorded at 1646 cm$^{-1}$ was strong in the SERS and redshifted some 10 cm$^{-1}$ with respect to the Raman spectrum, and the $\nu$(CH) vibration was the very weak band at 3051 cm$^{-1}$. Moskovits and Suh$^{18}$ assigned the $\delta$(CH) mode to the band at 1274 cm$^{-1}$. Consequently, the SERS spectrum in Figure 4(a) was the result of the competitive co-adsorption of aspartic acid in its anionic form and the fumarate anion produced by the reaction of adsorbate on the silver surface.

It is quite difficult to define a set of experimental conditions to reproduce the formation of the fumarate anion. That transformation must be catalyzed by active sites on the surface of the metal micelles whose existence depends on factors such as the aging of the colloid, which is quite difficult control. However, this particular kind of irreversible process is not unusual in SERS and can give rise to an incorrect interpretation if not detected.$^{10}$

**D$_2$O Silver Sols**

To our knowledge, no previous work on the vibrational spectrum of deuterated aspartic acid in either molecular or ionic forms has been published. In this type of molecular system it is important to realize that most of the vibrational modes are strongly coupled, so the assignments
proposed on the basis of only one internal coordinate are necessarily crude descriptions of the movement.

Figure 5 shows the Raman spectra of solid aspartic-$d_4$ acid (spectrum a) and a 1M solution of aspartic acid in $D_2O$ at pH 7 (spectrum b) and 14 (spectrum c) and the SERS spectrum on the $D_2O$ sol of a $10^{-3}$ M solution of aspartic acid at pH 7 (spectrum d).

![Figure 5](image)

**Figure 5.** The Raman spectra of solid aspartic-$d_4$ acid (spectrum a) and a 1M solution of aspartic acid in $D_2O$ at pH 7 (spectrum b) and 14 (spectrum c) and the SERS spectrum on the $D_2O$ sol of a $10^{-3}$ M solution of aspartic acid at pH 7 (spectrum d).
that they are assigned to bands at quite similar frequencies in the spectra of Gly-N-d$_3^-$ and α-Ala- 
N-d$_3^-$ 19,20 In the Raman spectra of Asp-d$_4$ and 
Asp-N-d$_3^-$ [Fig. 5(a,b)] there were two strong 
bands recorded at 818 and 820 cm$^{-1}$, respectively, 
which may be assigned to the r(ND$_3$) modes 
of these species. The band recorded at 932 cm$^{-1}$ 
in the SERS of the D$_2$O solution correlated with that 
recorded at 942 cm$^{-1}$ in the Raman of the alkaline 
solution in D$_2$O, as well as with that observed in 
the SERS obtained on the H$_2$O sol at 947 cm$^{-1}$ 
[Fig. 2(b)]; therefore, they must be assigned to the 
ν(C$\alpha$−COO) mode. This vibration was recorded in 
the Raman of the solid aspartic-d$_4$ at 895 cm$^{-1}$ 
and in the Raman of the anion at 930 cm$^{-1}$.

The SERS spectrum on D$_2$O showed a rather 
strong band at 976 cm$^{-1}$ that correlated with the 
one recorded in the Raman of the solution of the 
dianion at 981 cm$^{-1}$ and those recorded at about 
1000 cm$^{-1}$ in the spectra of the zwitterion and the 
anion, while no analogous bands were detected at 
similar frequencies in the spectra of the solutions 
in H$_2$O. The latter band was assigned to the 
ν(C$\alpha$−COO) mode, and it behaved systematically 
in the α-amino acids, increasing its frequency by 
the deuteration, which could be checked for the 
different deuterated species of glycine and α-alanine. 9 
Deuteration changes the coupling of that 
mode with others, mainly with ω(ND$_3$), and 
duces a blueshift that can be derived from the study 
of the force field of these molecules. For instance, in 
glycinate-N-d$_2^-$, a fundamental was computed at 
946 cm$^{-1}$ with a potential energy distribution 
(PED) contribution of 36% ν(C−COO), 20% ω(ND$_3$), 
and 16% β(CCN). This vibration corresponded to the 
band observed at 932 cm$^{-1}$ in the SERS of the 
D$_2$O solution. However, in the spectrum of the 
solution in H$_2$O the corresponding band was 
recorded at 911 cm$^{-1}$, the calculated frequency 
being 905 cm$^{-1}$, and the PED being in turn 50% 
ν(C−COO) and 20% β(CCO). In the Asp-N-d$_2^-$ 
the strong intensity of the band assigned to 
ν(C$\alpha$−COO) in the SERS was probably due to the 
contributions of the carboxylate and amino 
groups, which in turn may have interacted with 
the metal surface.

In the SERS on D$_2$O the medium band 
recorded at 1062 cm$^{-1}$ correlated with that at 1056 
cm$^{-1}$ in H$_2$O solution and was assigned to ν(CN). 
It can be correlated with those recorded at 1065, 
1043, and 1053 cm$^{-1}$ in the Raman spectra of the 
Asp-N-d$_2^-$ [Fig. 5(c)], Asp-N-d$_3^-$ [Fig. 5(b)], and 
Asp-d$_4$ [Fig. 5(a)] species, respectively. The weak 
band recorded at 1215 cm$^{-1}$ in the SERS and Ra-
man of the basic solution in D$_2$O was assigned to 
δ(ND$_3$), given that this frequency was very close to 
those observed in the spectra of many N-deuterated 
amines and amino acid anions. 16,20–25

The weak bands recorded in the SERS and 
Raman spectra of the different species at 1250 
and 1340 cm$^{-1}$ should be due to CH bendings. 
The broad and strong band observed in the SERS 
at 1392 cm$^{-1}$ must undoubtedly be assigned to 
ν$_s$(OCO) and correlated with those recorded at 
1420, 1414, and 1409 cm$^{-1}$ in the Raman spectra 
of the Asp-N-d$_2^-$, Asp-N-d$_3^-$, and Asp-d$_4$ species, 
respectively.

In the high frequencies region there was 
stretching of the ND$_3$ group observed at 2403 
cm$^{-1}$, while it appeared quite sharp at 2438 cm$^{-1}$ 
in the alkaline solution. Finally, the ν$_s$(CH$_2$) was 
recorded at 2930 cm$^{-1}$.

The analysis of the SERS spectrum of N-deu-
terated aspartic acid showed that the most en-
hanced bands were directly related to vibrations 
of the carboxylate and amino groups, which con-
firmed the adsorption of the anion through both 
functional groups. There were no characteristic 
bands of the fumarate ion observed in the sam-
ple obtained in D$_2$O.

Summarizing, the metal–biomolecule interac-
tion, and especially the metal–amino acid inter-
action, is a matter of great interest and has been 
the subject of numerous investigations. 26 The 
type of coordination of an amino acid can be quite 
variable, depending on the nature of the metal 
ions. In spite of the complexity of the SERS re-
sults and the particular molecule studied in this 
work, we proposed a series of conclusions related 
to the silver–aspartic system that we hope can be 
useful to better understand this type of problem 
in more complicated biomolecules like peptides or 
proteins. In addition, a series of questions com-
non to any SERS analysis was discussed.

REFERENCES

Raman Spectrosc 1990, 21, 43−48.
3. Lee, H. I.; Kim, M. S.; Suh, S. W. J Raman Spectro-
scop 1991, 22, 91−96.
4. Castro, J. L.; Montañez, M. A.; Otero, J. C.; Marcos, 
J. I. In VIIth European Conference on the Spectros-
copy of Biological Molecules; Carmona, P., Navarro,