

Surface-Enhanced Raman Spectroscopy of Amino Acids and Nucleotide Bases Adsorbed on Silver

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Abstract: The SERS spectra of amino acids (glycine, α - and β -alanine, 6-aminocaproic acid, and *o*- and *m*-aminobenzoic acids (OABA and MABA)) and nucleic acid bases (adenine, cytosine, and uracil) adsorbed on the surface of silver colloid particles are reported. We interpret the SERS spectra of the aliphatic amino acids as indicating that both the amino and carboxylate groups are bound to the silver colloid surface. By analyzing the relative band intensities, we propose the geometry of each of the amino acids on the surface. The SERS spectra of OABA at high and low pH are very similar. In contrast the SERS spectra of MABA at high and low pH differ markedly, signaling a change of surface geometry for the molecule as a function of pH. Likewise the orientations of adenine, cytosine, and uracil on the surface of Ag colloid have been determined on the basis of their SERS spectra and the SERS spectra of the N-deuterated compounds.

The vibrational spectra of the amino acids have been extensively studied both in solution¹⁻³ and in their crystalline states.⁴⁻⁹ X-ray studies show that crystalline aliphatic amino acids adopt a zwitterionic structure which is held together by a complicated network of hydrogen bonds.¹⁰ In solution, each amino acid can exist in three possible forms: the anion (NH_2RCOO^-), the dipolar (zwitter-) ion ($^+\text{H}_3\text{NRCOO}^-$), and the cation ($^+\text{H}_3\text{NRCOOH}$). Vibrational assignments for the three forms of glycine in water have been well documented.³ The vibrational frequencies of these three forms are slightly different from one another. Raman spectra of metal-amino acid complexes in aqueous solution have also been reported,¹¹ and the additional vibrational data which the complexes yield will be very useful in understanding the properties of amino acids adsorbed on metal surfaces.

m-Aminobenzoic acid has a predominantly zwitterionic structure in aqueous solution, in contrast with the ortho and para isomers^{12,13} which exist primarily as the molecular structures. The different structure of *m*-aminobenzoic acid in aqueous solution results in different physical properties, such as solubility and electrical conductivity, from those of the para and ortho isomers.^{14,15}

The vibrational study of the amino acids on metal surfaces is of particular interest, since both the amino and carboxylate groups are possible points of attachment to the surface.

In this paper, we report the SERS spectra of glycine, α - and β -alanines, 6-aminocaproic acid, and *o*- and *m*-aminobenzoic acids. The SERS spectrum of *p*-aminobenzoic acid has been discussed previously.¹⁶ In addition the SERS spectra of adenine, cytosine,

and uracil are presented.

Experimental Section

Aqueous silver colloid was prepared by reducing silver nitrate with sodium borohydride: A sodium borohydride solution (60 mL of 2×10^3 M) was mixed with a silver nitrate solution (22 ± 2 mL of 1×10^3 M) with both solutions chilled to ice temperature. D_2O colloids were made in exactly the same fashion, substituting D_2O (98%) for water. Amino acid solutions were prepared by dissolving small amounts of these solids in water and NaOH solution, respectively. Adenine, cytosine, and uracil solutions were prepared by dissolving small amounts of these solids in water. N-Deuterated species of adenine, cytosine, and uracil were prepared by dissolving the molecules in D_2O to undergo isotopic exchange and evaporating to dryness. This procedure was repeated 3 times. The adsorbate was introduced into the colloid by adding one drop of the above solutions to approximately 1.5 mL of colloid solution. When adsorbate was added, the color of the colloid changed from yellow to blue. Spectra were recorded on a SPEX 1400 Series monochromator equipped with photon counter and interfaced to a Tektronix 4052 computer. These are shown in Figures 1-8. Samples were excited with a Spectra Physics Model 165 Ar ion laser.

Discussion

In deriving orientational information from the SERS spectra we will make use of the so-called SERS surface selection rule²⁰ which, stated briefly, predicts that the vibrational bands which draw their intensity from the Raman polarizability component α_{zz} will be most intense (z being the surface normal) and, indeed, the only bands present in the spectrum if laser excitation well to the red of the surface plasmon frequency is used. When (as in our case) this condition is not fully complied with, then one would also see bands deriving their intensity from the α_{xz} and α_{yz} components, albeit with less intensity. The distinction between this surface selection rule and the more familiar infrared surface selection rule should be borne in mind. In the latter one only sees bands whose transition dipoles have a z component; hence vibrations whose normal coordinates are directed normal to the surface are "allowed"; others are "forbidden". Because many Raman active modes do not transform as displacements they cannot be spoken of as being "directed", making the Raman selection rule less easily visualized and hence more subtle.

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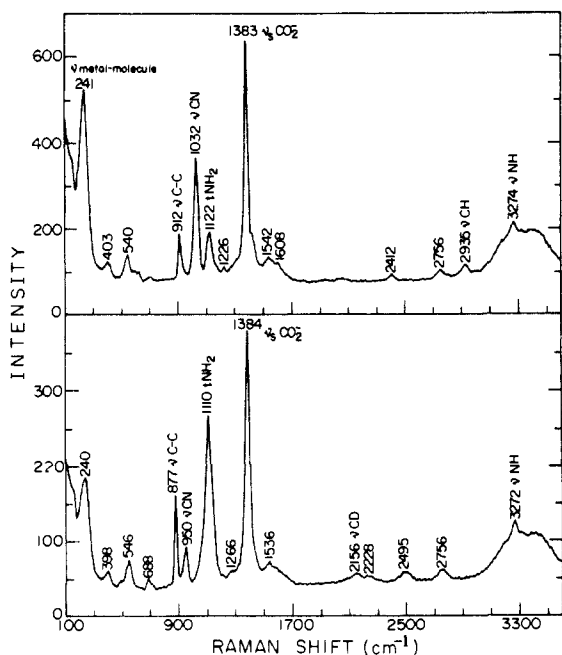


Figure 1. Upper: SERS spectrum obtained after adding a drop of glycine solution to an aqueous silver sol, excited with the 514.5-nm argon laser line. Lower: same as above but using glycine-*d*₅.

Nevertheless one may obtain powerful insights by considering, intuitively, the possible connection between the atomic motion involved in a vibration and the magnitude of the resulting Raman polarizability components. Consider, for example, a benzene molecule adsorbed flat on a metal surface. Its two *a*_{1g} modes (*ν*₁ and *ν*₂) are the only ones to which α_{zz} contributes. The components α_{xx} and α_{yy} also contribute to the intensity of these modes. In *ν*₁ (992 cm⁻¹) it is almost exclusively the carbon atoms which move in a symmetric "breathing" vibration; *ν*₂ (3056 cm⁻¹) corresponds to a "breathing" motion of the hydrogen atoms. In both cases the nuclear motion is restricted to the *x-y* plane, and yet α_{zz} , which expresses the change in the molecular polarizability perpendicular to this plane, is nonvanishing.

Since $\alpha_{xx} = \alpha_{yy}$ for benzene, by symmetry, one can relate the ratio α_{zz}/α_{xx} to the depolarization ratio, ρ_s , which is defined as

$$\rho_s = \frac{3\gamma^2}{45\bar{\alpha}^2 + 4\gamma^2}$$

where

$$\bar{\alpha} = \frac{1}{3}(\alpha_{xx} + \alpha_{yy} + \alpha_{zz})$$

$$\gamma^2 = \frac{1}{2}[(\alpha_{xx} - \alpha_{yy})^2 + (\alpha_{yy} - \alpha_{zz})^2 + (\alpha_{zz} - \alpha_{xx})^2]$$

through the expression

$$\rho_s = (1 - \alpha_{zz}/\alpha_{xx})^2 / (8 + 4\alpha_{zz}/\alpha_{xx} + 3\alpha_{zz}^2/\alpha_{xx}^2)$$

This expression, being quadratic, yields two values of α_{zz}/α_{xx} for any value of ρ_s .

For the two *a*_{1g} modes of benzene, for example, for which (liquid phase) depolarization ratios are 0.02 and 0.2, respectively, one calculates $\alpha_{zz}/\alpha_{xx} = 1.7$ and 0.53 for *ν*₁ and $\alpha_{zz}/\alpha_{xx} = 7.2$ and -0.21 for *ν*₂.

Intuition dictates that one choose the values which are less than unity, however, since it is unlikely that the change in the component of the molecular polarizability perpendicular to the plane containing the nuclear motion should exceed the change in the magnitude of the in-plane components.

One notes that the ratio α_{zz}/α_{xx} is about 2.5 times larger for the *ν*₁ vibration than for *ν*₂. This is precisely what intuition suggests. The greatest contribution to the α_{zz} component of the polarizability of benzene comes from the π -electrons. It seems natural that *ν*₁, which perturbs the carbon ring directly, should affect the α_{zz} component more profoundly than *ν*₂ which involves mainly hydrogen motion.

On going from solution to the surface one expects the intensity of *ν*₂ to be reduced with respect to that of *ν*₁, roughly by a factor of 6 (i.e., 2.5²). (One sees a somewhat greater reduction because *ν*₂, having a greater frequency, cannot benefit as greatly from the simultaneous resonance of the incident and Raman-shifted fields with the surface plasmon of the underlying surface.)

The situation would be different if the benzene ring could somehow be caused to "stand up" on the surface. In that case the C-H vibration (*ν*₂) would be intense since a much larger Raman polarizability component of the molecule would become the α_{zz} component. This is precisely what is seen with the phthalate ion which stands up on the surface as compared with the isophthalate ion which lies down.²⁴ In the former the C-H vibrations

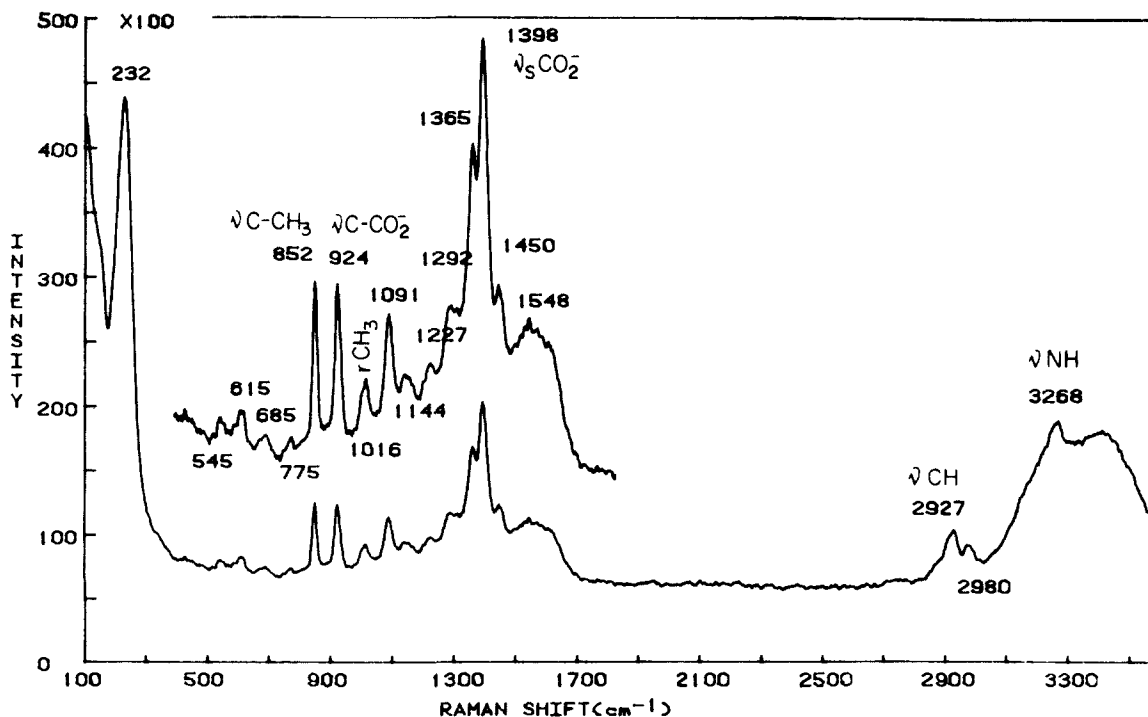


Figure 2. As in Figure 1 but for α -alanine.

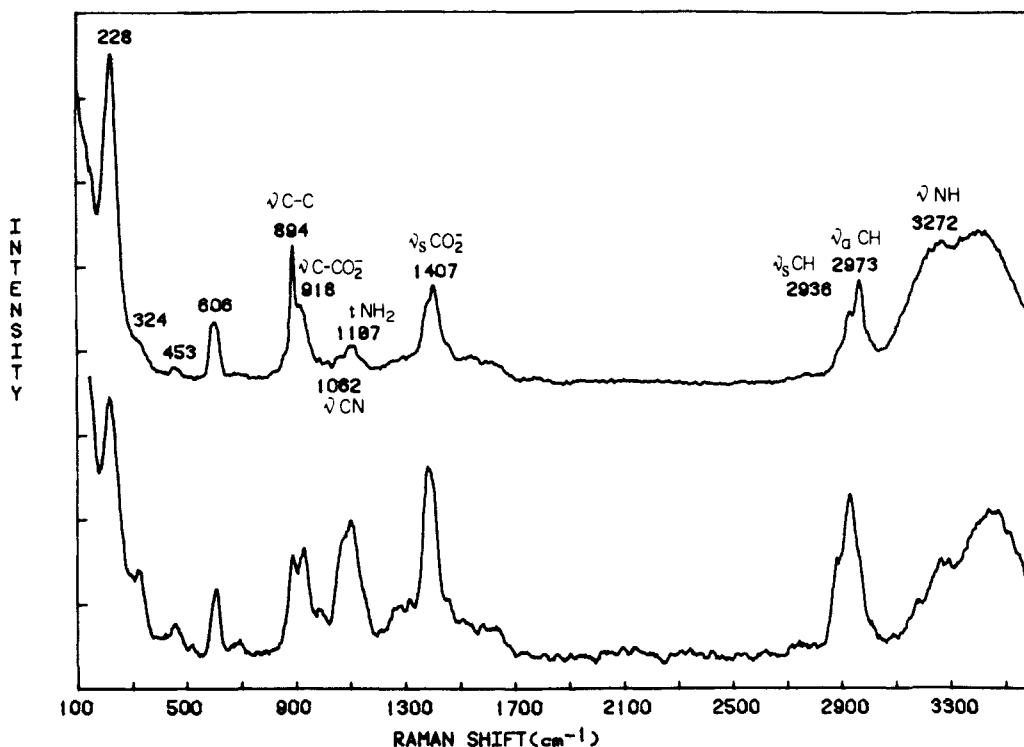


Figure 3. As in Figure 1 but using β -alanine solution without NaOH (upper) and with NaOH (lower).

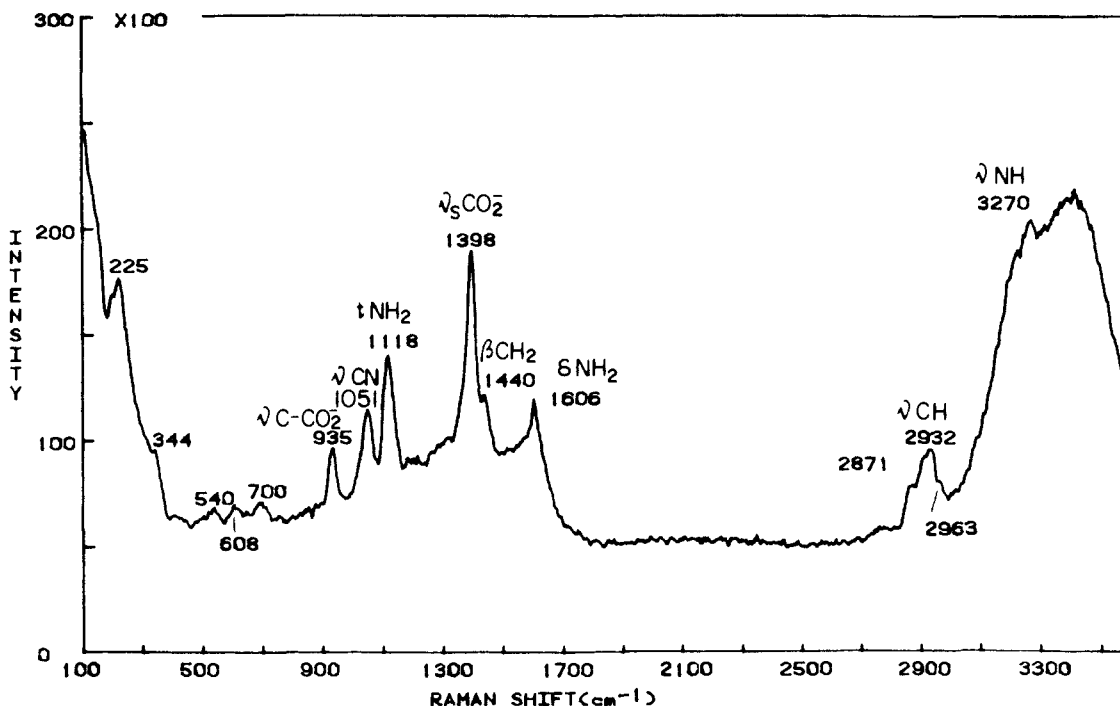


Figure 4. As in Figure 1 but for 6-aminocaproic acid.

are strong; in the latter they are very weak.

In the following we will assume that C-H vibrations have only weak components of Raman polarizability perpendicular to the plane containing the H nuclear motion. Their presence or absence in the SERS spectrum will therefore be assumed to signal, respectively, a molecular orientation with C-H bonds disposed, at least, partially along the normal to the surface and entirely parallel to the surface.

Amino Acids. Glycine ($\text{NH}_2\text{CH}_2\text{COOH}$). The SERS spectrum of glycine is very different from that of the aqueous solution. For example, in the solution spectrum bands belonging to ν_{CH} , $\nu_{\text{s,COO}^-}$,

CH_2 wagging, and $\nu_{\text{C-C}}$ which come at 2976, 1413, 1332, and 900 cm^{-1} , respectively, are strong while bands due to CN stretching and NH_2 twisting at 1033 and 1122 cm^{-1} , respectively, are weak. By contrast, in the SERS spectrum (Figure 1) the strongest band at 1383 cm^{-1} is due to $\nu_{\text{s,COO}^-}$. The carboxyl group is clearly ionized on the surface because the band at 1383 cm^{-1} belongs to a carboxylate group ($-\text{COO}^-$), and there is no indication of the 1600- cm^{-1} vibration due to the C=O portion of the carboxyl group ($-\text{COOH}$). Other strong bands are observed at 241 and 1032 cm^{-1} . The intensity of the C-C stretching at 912 cm^{-1} is weak in the SERS spectrum but strong at 900 cm^{-1} in the solution spectrum. Also, there is at least a 10- cm^{-1} blue shift in the frequency of this band in the SERS spectrum with respect to its

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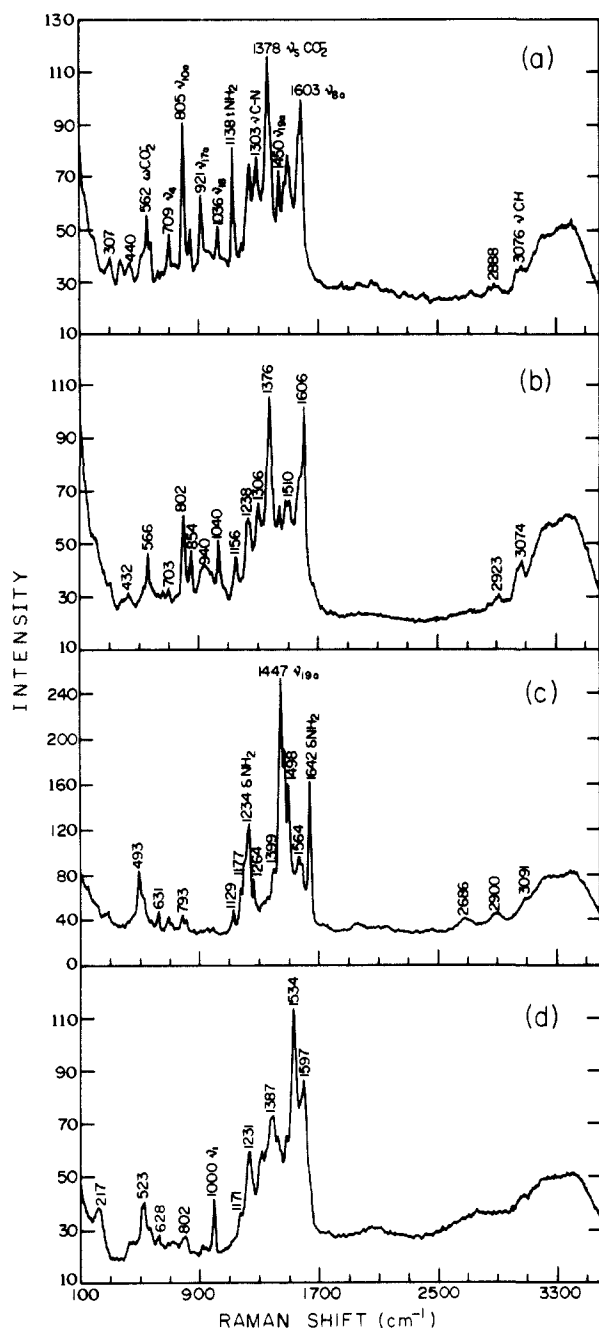


Figure 5. (a) SERS spectrum obtained with low pH OABA excited with 514.5-nm Ar⁺ laser light. (b) Same as above but with high pH OABA. (c) As above but with high pH MABA. (d) As above but with low pH MABA.

frequency in the solution spectrum. This is reminiscent of the shift observed in zinc nitrate–glycine solution.³ In that system at low pH (pH 2), ν_{C-C} comes at 902 cm^{-1} , which is very close to that of pure glycine solution, but at higher pH (pH 5), its frequency rises to 918 cm^{-1} . Therefore, it was concluded that glycine at high pH is bonded to the (Zn) metal ion in a bidentate fashion. We ascribe the blue shift of ν_{C-C} in the SERS spectrum to the same effect. In our system, this means that the carboxylate and amino groups are both attached to the surface. There is other evidence which points to this geometry. For example, the $\nu_{s,CH}$ band of the anionic, zwitterionic, and cationic forms comes at 2935, 2968, and 2975 cm^{-1} , respectively.³ In the SERS spectrum, $\nu_{s,CH}$ is found at 2935 cm^{-1} , as in the anionic form. The anionic form of glycine is the preferred form on the surface when both the carboxylate and amino groups are attached, since only in this form are both ends good Lewis bases. The fact that the molecule adsorbs on the silver colloid surface as the anion does not preclude a geometry in which the COO^- group is on the surface, while the

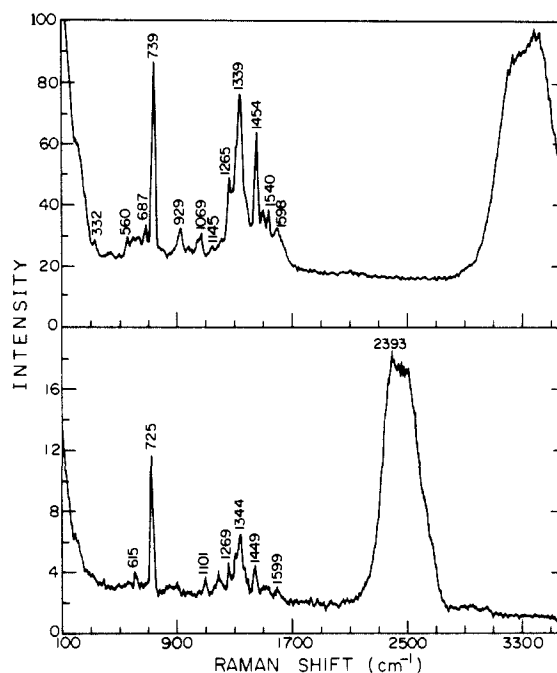


Figure 6. Upper: as in Figure 1 but with adenine (excited with 488-nm Ar⁺ laser light). Lower: as above but with N-deuterated adenine added to aqueous silver sol prepared in 98% D₂O.

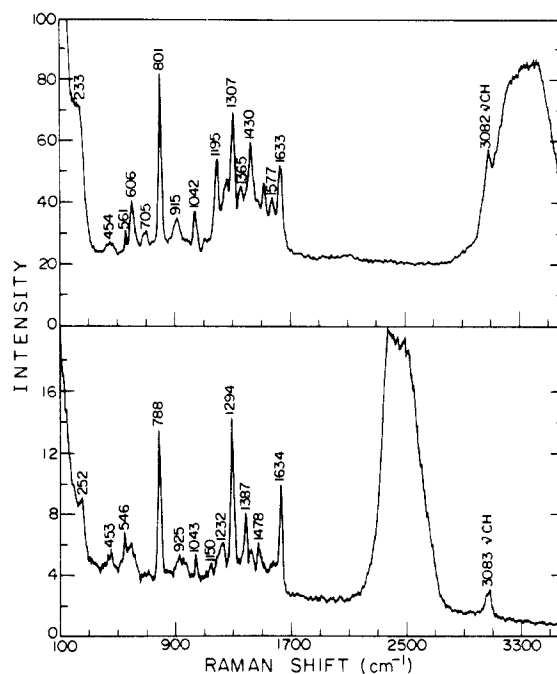


Figure 7. As in Figure 6 but for cytosine (upper) and N-deuterated cytosine in 98% D₂O silver sol (lower).

amino group is in solution. We have other evidence to exclude this, however. The strong band due to ν_{CN} at 1032 cm^{-1} in the SERS spectrum can only be explained by assuming that the amino group is attached to the surface; this band is weak in the solution spectrum. The intensities of bands related to the amino group vibrations are relatively strong. A medium strong band at 1122 cm^{-1} is possibly due to NH_2 twisting or NH_3^+ rocking. We assigned this band to the twisting mode of NH_2 because the SERS spectrum at high pH (with NaOH) is exactly the same as that at low pH (without NaOH). At high pH, the anion form will undoubtedly be dominant. Further support for this surface geometry is the band at 3274 cm^{-1} , which is clearly due to NH_2 stretching. The frequency of NH_3^+ stretching has a very different value (about 2980 cm^{-1}). The broad background in this spectral region is due to water. Therefore, the evidence is strong that both

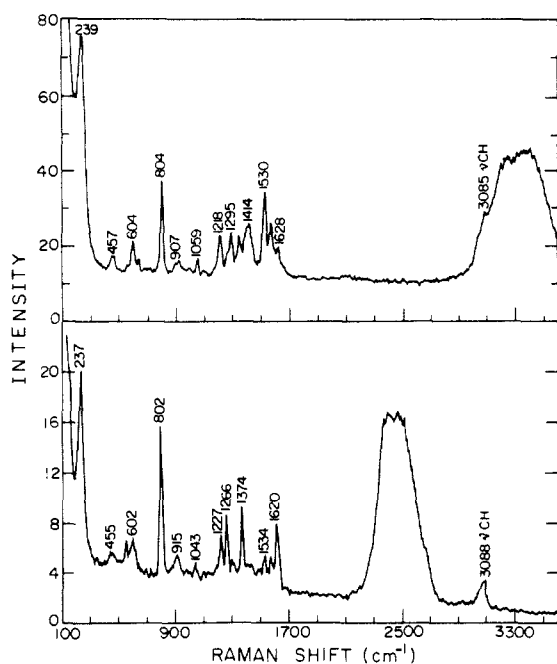


Figure 8. As in Figure 6 but for uracil (upper) and N-deuterated uracil in 98% D₂O silver sol.

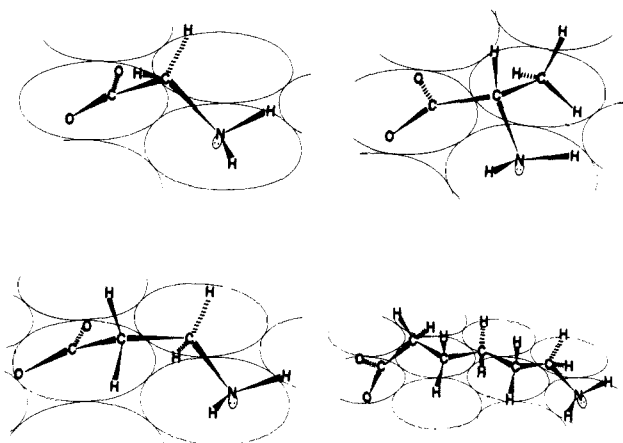


Figure 9. Proposed surface geometry of (clockwise from upper left) glycine, α -alanine, 6-aminocaproic acid, and β -alanine, adsorbed on the surface of a silver sol particle.

the carboxylate and amino groups are attached to the silver surfaces.

The relative position of the methylene group has not yet been addressed. The intensity of ν_{CH} is rather weak in the SERS spectrum (in contrast to the solution spectrum). One possible cause of this is that the methylene group could be relatively far from surface. The geometry shown in Figure 9 has this required property and is consistent with the other spectral results discussed above.

There still remain several unassigned bands in the SERS spectrum. The strong band at 241 cm^{-1} is attributed to the metal-molecule vibration. COO^- rocking, wagging, and bending modes are observed between 500 and 700 cm^{-1} . The region between 1500 and 1650 cm^{-1} is complicated. The H_2O deformation, NH_2 deformation, and COO^- antisymmetric stretching modes all lie in this region.

The weak band at 2414 cm^{-1} is probably a combination band of ν_{CN} and $\nu_{\text{s,COO}^-}$, while the band at 2756 cm^{-1} is an overtone of $\nu_{\text{s,COO}^-}$.

The SERS spectrum of glycine- d_5 in Ag colloid solution, with water as the solvent, is shown in Figure 1. As a result of exchange with the hydrogens of water, glycine- d_5 becomes $\text{NH}_2\text{CD}_2\text{COO}^-$ in solution. Consequently the band in the SERS spectrum corresponding to CH is replaced by one due to CD near 2156 cm^{-1} ,

while the band at 3272 cm^{-1} corresponding to ν_{NH_2} remains. As expected, the frequencies corresponding to COO^- group vibrations are not shifted. For example, the strongest band, due to $\nu_{\text{s,COO}^-}$, is found at 1384 cm^{-1} . This value is the same as that of glycine within experimental error. The frequencies of $\nu_{\text{C-C}}$ at 877 and ν_{CN} at 950, and NH_2 twisting at 1110 cm^{-1} are red shifted and their relative intensities are also changed, with the relative intensities of NH_2 twisting and CN stretching changing upon deuteration.

It is interesting to note that at the pH values studied (near pH 7) glycine exists in solution as a zwitterion, while on the silver surface it is found to be wholly in the anionic form normally adopted a high pH. There may be several reasons for this. The first, implied above, is that only by remaining in the anionic form will the molecule maintain the amino end as a good surface bonding group. In the zwitterionic form that group becomes NH_3^+ which is not a good Lewis base. Alternatively one may deduce from our observation that the effective pH at the surface of the colloidal particle is higher than in solution. (It would have to be at least two pH units higher to account for the absence of the zwitterion). This is not an unreasonable interpretation since it is known that the surface of the colloidal particle is positively charged and would therefore exclude H^+ ions to a great extent from its immediate vicinity.

α -Alanine ($\text{NH}_2\text{CHCH}_3\text{COOH}$). The structure of α -alanine is the same as that of glycine except that one hydrogen of the methylene group is replaced by a methyl group whose presence may change the disposition of the molecule on the Ag surface when compared to that of glycine. Likewise, a slight change in geometry may alter the relative intensities of vibrational modes. The SERS spectrum of α -alanine (Figure 2) is indeed different from that of glycine in several ways. There are two C-C stretching bands: the C- CH_3 stretching at 852 cm^{-1} and the C- COO^- stretching at 924 cm^{-1} . The intensity of these two bands is almost the same. In the solution Raman spectrum, the intensity of $\nu_{\text{C-COO}^-}$ at 921 cm^{-1} is very weak but that of $\nu_{\text{C-CH}_3}$ at 849 cm^{-1} is very strong.

The intensities of the amino group vibrations are very different from those of glycine. The intensity of the twisting mode of NH_2 near 1144 cm^{-1} is relatively weak, while the intensity of NH_2 stretching at 3268 cm^{-1} is relatively strong. The band corresponding to CN stretching (1091 cm^{-1}) has medium intensity.

There are several bands due to the methyl group. The band at 1016 cm^{-1} is due to CH_3 rocking. The symmetric and antisymmetric stretching vibrations of the CH_3 group come at 2927 and 2980 cm^{-1} , respectively. In the solution Raman spectrum, ν_{CH} of the methyne group is very strong (2960 cm^{-1}), but in the SERS spectrum, the contribution of this vibration to the CH stretching manifold seems very small.

The bands corresponding to COO^- vibrations between 500 and 700 cm^{-1} are relatively weak.

These vibrational data suggest that the surface geometry of α -alanine is clearly different from that of glycine. Let us now deduce a possible geometry of α -alanine on the silver surface. We interpret the relative strength of the bands corresponding to the CH_3 group, as evidence that that group must be close to the surface. Likewise the weak contribution from the methyne group in the CH-stretching region implies that it is far from the surface. As the relative band intensities of $\nu_{\text{C-CH}_3}$, $\nu_{\text{C-COO}^-}$, and $\nu_{\text{C-NH}_2}$ are almost the same, those groups are deployed more or less equivalently with respect to the surface. Finally, the NH bonds must stand in a more perpendicular fashion to the surface as compared to those of glycine.

On the basis of these arguments, α -alanine is proposed to lie on the surface as shown in Figure 9.

β -Alanine ($\text{NH}_2\text{CH}_2\text{CH}_2\text{COOH}$). The Raman spectrum of β -alanine is expected and is found to be similar to that of glycine except in the C-C-stretching region. The relative band intensities differ, however. Two SERS spectra of β -alanine are shown in Figure 3. The upper spectrum of Figure 3 was taken after adding one drop of β -alanine solution which was dissolved in water to 2 mL of silver colloid solution, and the lower spectrum of Figure 3 was taken after adding one drop of β -alanine solution whose

pH was adjusted to pH 11 by adding NaOH to 2 mL of silver colloid solution. Systematic changes were found in the relative band intensities of the two spectra. For example, the intensities of the bands at 228 cm^{-1} and $\nu_{\text{C-COO}^-}$ at 918 cm^{-1} anticorrelate. When the one is strong, the other is weak. The same relationship was found with $\nu_{\text{C-C}}$ at 894 cm^{-1} and the NH_2 -twisting band near 1107 cm^{-1} .

The intensity of the NH_2 -twisting band in the SERS spectrum of alkaline β -alanine is stronger than that in the corresponding SERS spectrum at low pH. β -Alanine has a zwitterionic structure when dissolved in the absence of base. We interpret the intensity variations as a result of increasing pH as follows: In the "low pH" case, only the carboxylate group bonds to the surface. Initially the $-\text{NH}_3^+$ group will interact only weakly with the surface, until it loses a H^+ ion. The band at 3272 cm^{-1} , due to NH_2 stretching, provides evidence that $-\text{NH}_3^+$ groups ultimately become NH_2 on the surface. However, the surface site chosen does not ensure that both the carboxylate and the amino groups will find favorable surface bonding sites, since only the carboxylate group is suitable for binding to the surface initially; hence the relative intensity of NH_2 vibrations will be lower. In the "high pH" solution, on the other hand, β -alanine already has the anionic structure. Hence, both groups will be available for binding to the surface simultaneously. In this case, all (or most) of the molecules will seek surface sites at which both groups may bind to the surface.

The CH-stretching vibrations in the SERS spectrum of this molecule are very strong. One interesting observation is that the intensity of antisymmetric CH-stretching vibration is greater than that of the symmetric stretch. This is unusual and can only be understood if the methylene group is so disposed with respect to the surface that the line bisecting the two CH bonds is more or less parallel to the surface.

When both the COO^- and the NH_2 groups are bound to the surface, the molecule must adopt a gauche conformation as in Figure 9; although unusual when viewed from the point of view of what exists in solution, this conformation allows the most benefit to be gained from making the surface bonds. This surface geometry accounts for the aforementioned band intensity changes in terms of the nearness and relative disposition of the two bonding groups with respect to the surface. For example, with the $-\text{NH}_2$ group close to the surface, the C-C bond is less perpendicular to it (Figure 9), and its intensity is expected to decrease, while that of ν_{CN} is expected to increase. This behavior is clearly seen in the SERS spectra.

One band near 324 cm^{-1} observed in the SERS spectrum at high pH is somewhat mysterious. One possible assignment is to a pseudocyclic breathing mode similar to that found in the SERS spectrum of succinic acid.¹⁷

6-Aminocaproic Acid ($\text{NH}_2(\text{CH}_2)_5\text{COOH}$). The SERS spectrum of 6-aminocaproic acid (Figure 4) is similar to that of glycine, differing mainly in the relative intensities of some bands. The intensity of the NH_2 -twisting mode at 1118 cm^{-1} is stronger than those of $\nu_{\text{C-COO}^-}$ at 935 cm^{-1} and ν_{CN} at 1051 cm^{-1} . There is no band corresponding to the vibration of the central C-C. A sharp band is observed at 1606 cm^{-1} (a sharp, medium-intensity band in this region was also sometimes observed in the SERS spectrum of glycine.) This band may be due to NH_2 deformation. The CH-stretching bands are broad, with a few sharp features. The maximum is at 2932 cm^{-1} , which corresponds to the frequency of symmetric stretching of CH. The relative intensity of CH is low considering that the molecule possesses six methylene groups. The weak shoulder at 1440 cm^{-1} is due to CH_2 bending. The bands between 400 and 800 cm^{-1} are weak.

If 6-aminocaproic acid were all-trans, both bonding groups would point toward the surface. But such a geometry would place two of the methylene groups too close to the surface to allow the bonding groups to remain attached without strain. The all-trans conformation may, therefore, be ruled out. A clue to the surface geometry may come from the fact that interior C-C vibrations are not seen and that relatively weak CH vibrations are observed. This may result from a change in the surface geometry of 6-aminocaproic acid from the all-trans form (perhaps as shown in

Figure 9), which would somewhat remove the central C-C bonds and most C-H oscillators from the surface.

***o*-Aminobenzoic Acid (OABA).** Both the solution and solid Raman spectra of OABA were very weak. Vibrational mode assignments were made by comparison with published data for disubstituted benzenes¹⁸ and isophthalic acid.¹⁹

The SERS spectra of OABA at low (Figure 5) and high (Figure 5) pH are, more or less, the same. However, the relative intensities of some bands are changed. Benzene ring vibrations dominate the SERS spectrum. These are ν_{8a} at 1603 , ν_{8b} at 1583 , ν_{19b} at 1483 , ν_{19a} at 1450 , at ν_{18} at 1036 cm^{-1} , all in-plane benzene ring vibrations. The out-of-plane benzene ring vibrations are relatively strong. The strong band at 805 cm^{-1} and a medium band at 853 cm^{-1} are probably due to ν_{10a} and ν_{10b} . The band at 709 cm^{-1} is due to ν_4 . The other out-of-plane vibration of 321 cm^{-1} is due to ν_{17a} . In the high pH spectrum, the intensities of out-of-plane vibrations are decreased, while the intensity of CH is increased in relation to the other bands.

Besides the benzene ring vibrations, there are several bands in the SERS spectrum related to the vibrations of carboxylate and amino groups. The strongest band at 1378 cm^{-1} is due to $\nu_{\text{s,COO}^-}$ and the band at 562 cm^{-1} is due to COO^- wagging. The strong band at 1138 cm^{-1} is due to NH_2 twisting and has a shoulder at 1156 cm^{-1} which is dominant in the high pH spectrum attributed to ν_{9a} . The band at 1250 cm^{-1} consists of two components, one at 1238 cm^{-1} and the other at 1253 cm^{-1} . The former, due to NH_2 deformation, is strong in the high pH SERS spectrum. The medium-intensity band at 1303 cm^{-1} is attributed to $\nu_{\text{C-NH}_2}$. This band will be discussed in some detail in the section on nucleotide bases.

The SERS spectrum of OABA is probably due to two, coexisting surface forms of the molecule. In one the benzene ring is flat on the surface, while in the other the benzene ring is slightly tilted with respect to the surface. The SERS spectrum of the former species would contain strong out-of-plane vibrations, while the SERS spectrum of the latter is expected to have intense CH-stretching vibrations. For a benzene ring lying flat on the surface, the intensity of ν_{CH} would be almost zero, since in that case the CH-stretching vibrations do not have contributions from α_{zz} , α_{xz} , and α_{yz} Raman polarizability components which would result in strong SERS intensity (where z is the surface normal). However, the out-of-plane vibrations do have large contributions from these Raman polarizability components. With the benzene ring slightly tilted from the surface, the CH-stretching vibration would acquire some contribution from the required Raman polarizability components, and simultaneously, the magnitude of these components will decrease for the out-of-plane vibrations. The spectral data imply that in low pH samples, the flat species dominates, while in the high pH samples, the concentration of the tilted species increases.

***m*-Aminobenzoic Acid (MABA).** SERS spectra of MABA at high pH (with NaOH) and low pH (without NaOH) differ markedly. Spectra of the low pH samples were hard to reproduce and often yielded poorly resolved bands on top of a broad background in the 1100 – 1600-cm^{-1} range. One of the best spectra obtained is shown in Figure 5. For the high pH samples, the SERS spectra were very easily and reproducibly obtained.

In the SERS spectrum of high pH samples (Figure 5), the strongest band at 1447 cm^{-1} is due to ν_{19a} . This is a mixed mode to which both ring stretching and CH deformation contribute. This mode is also the strongest band in the SERS spectrum of PABA.¹⁶ There are two shoulders at 1471 and 1498 cm^{-1} . One of these bands is probably due to ν_{19b} . A weak shoulder at 1177 cm^{-1} is probably due to ν_{9a} . The strong band at 1642 cm^{-1} is probably due to NH_2 deformation. (Its frequency is too high for it to be a benzene ring vibration.) The band at 1129 cm^{-1} is due to NH_2 twisting, while the strong band at 1234 cm^{-1} , also observed in the SERS spectra of other aminobenzoic acids but not in those of phthalate, isophthalate, and terephthalate ions, is probably related to an amino group vibration. Accordingly we assign it to a NH_2 deformation. $\nu_{\text{C-NH}_2}$ near 1310 cm^{-1} is very weak, while the band near 500 cm^{-1} consists of two bands: ν_{16a} and ν_{16b} .

Usually, ν_{s,COO^-} is one of the stronger bands in the SERS spectra. In the spectrum of the high pH samples of MABA it is very weak. The weak shoulder at 1399 cm^{-1} is assigned to this vibration. This implies that the carboxylate group is lying with its plane almost entirely flat on the surface. By contrast ν_{s,COO^-} is strong in the SERS spectra of low pH samples. The frequency of the strongest band at 1534 cm^{-1} is close to that of ν_{a,COO^-} . However, ν_{19a} is another possible assignment for this band. We prefer the former since the observed frequency is too high when compared with the value of ν_{19a} obtained from the IR data.¹⁵ The band at 1597 cm^{-1} is due to ν_{8a} , and the sharp band at 1000 cm^{-1} is due to ν_1 . This mode is the symmetric ring-breathing vibration and is expected to be strong when the benzene ring is standing up on the surface. In the SERS spectrum of high pH samples, this band (at 977 cm^{-1}) is very weak.

In this case we conclude that MABA in the low pH samples adopts a geometry which is either standing up or at least tilting with respect to the surface, while in the high pH samples the molecule lies almost flat on the surface. This is consistent with the fact that MABA has the zwitterionic form at low pH and the anionic form at high pH. The latter has a planar structure which may bind to the surface through the π -electrons of the phenyl ring. In low pH samples, the carboxylate group bonds to the surface. The $-NH_3^+$ would, at least initially, prevent the molecule from lying flat.

Nucleotide Bases (Adenine, Cytosine, and Uracil). The SERS spectra of three of the bases produced in the hydrolysis of ribonucleic acids (RNA) (adenine, cytosine, and uracil) are shown in Figures 6–8. Guanine did not yield a SERS spectrum. This is probably due to its very low solubility in water.²¹

The SERS spectra are very different from the Raman spectra of the corresponding solids. In the Raman spectra of the solids, there are strong bands at 1335 and 728 cm^{-1} for adenine, at 1290 and 780 cm^{-1} for cytosine, and at 1235 and 785 cm^{-1} for uracil.²²

Adenine. The SERS spectrum of adenine at a silver electrode was reported by Kogline et al.²³ This spectrum is more or less the same as the one we obtain with silver sols (Figure 6), except that the strongest band in Kogline's spectrum comes at 250 cm^{-1} , and a weak band at 1454 cm^{-1} in his spectrum is a strong band in ours (Figure 6). Kogline et al. assigned the strong bands at 736 and 1337 cm^{-1} as the primarily skeletal vibrations of the adenine ring. These frequencies agree well with ours, within experimental error. Figure 6 also shows strong bands at 739 , 1338 , and 1454 cm^{-1} . One might conclude that the band at 736 cm^{-1} corresponds to the band at 728 cm^{-1} in the Raman spectrum of the solid, which is assigned to the ring-breathing vibration of adenine.²¹ In solution, and in the solid, this mode usually corresponds to a strong band. In the SERS spectrum, on the other hand, a high intensity is only expected for this band when the ring is standing up or is tilted with respect to the surface, as in the SERS spectrum of the phthalate ion which is standing up on the surface.²⁴ The high intensity of this band in the SERS spectrum of adenine adsorbed on Ag might force one to conclude that this molecule stands erect on the metal surface as well. Such a conclusion could not be supported, however, by the absence of a CH-stretching band at about 3080 cm^{-1} , which is also a characteristic of a molecule "standing" on the surface, because both the ring-breathing vibration and the CH-stretching vibrations make strong in-plane contributions to the Raman polarizability tensor. Unfortunately, Kogline et al. did not show the CH-stretching region in their spectrum, and we cannot, therefore, deduce the geometry of adenine at their silver electrode. This discrepancy may be resolved in one of two ways. The first is to assume that the band at 728 cm^{-1} in the solid Raman spectrum is not due to the ring-breathing vibration. The other is to postulate that the band 739 cm^{-1} in the SERS spectrum (Figure 6) is coincidentally close in frequency to the ring-breathing vibration but that, in fact, it belongs to another vibration. A possible candidate is the band near 730 cm^{-1} assigned by Speca et al.²⁵ to a coupled NH_2 deformation and ring vibration in the IR study

of adenine-metal complexes. This frequency changes from 714 to 740 cm^{-1} depending upon the complex.

Adenine and cytosine have an amino group which, in these molecules, is thought to be nearly planar as a result of resonance structures which place some double bond character in the C–N bond of these molecules. This partial double bond character is also manifested in the frequency of the C–N-stretching modes of these molecules which occurs near 1050 cm^{-1} for aliphatic compounds but near 1310 cm^{-1} for aromatics in which similar resonance structures contribute. The SERS spectra of adenine and cytosine have bands associated with the amino group in this region. A strong band at 1307 cm^{-1} for cytosine is mainly due to ν_{C-NH_2} , and a weak shoulder at 1317 cm^{-1} for adenine may also be due to ν_{C-NH_2} . The fact that this band is weak for adenine adsorbed on the surface would not be unexpected for a molecule lying flat on the surface. This is, in fact, our conclusion. For completeness, we assign the band at 1265 cm^{-1} to the asymmetric out-of-plane deformation of $-NH_2$, analogous to bands observed in the SERS spectra of the aminobenzoic acids, while the strong band at 1454 cm^{-1} in the SERS spectrum of adenine is probably a ring vibration.

Cytosine and Uracil. The SERS spectra of cytosine and uracil are similar. Each has its strongest band near 802 cm^{-1} , and there are two similar bands of medium intensity between 400 and 610 cm^{-1} . Both spectra exhibit strong CH stretching bands at about 3080 cm^{-1} . This similarity is due to the same ring structure and a similar geometry on the surface.

Assignment of the strongest band is not easy. Two, or even three, vibrational modes are possible. Unlike adenine, we cannot eliminate the contribution of the ring-breathing vibration to this strongest band, since the intensity of the CH-stretching vibration in both SERS spectra implies that in both molecules the pyrimidine ring is perpendicular, or at least tilted, with respect to the surface. Hence, a strong ring-breathing vibration is expected. However, a frequency of 802 cm^{-1} is somewhat high for it to correspond to the strong band near 780 cm^{-1} in the Raman spectrum of the solid. At any rate it is not even certain that the strong band near 780 cm^{-1} in the Raman spectrum of the solid is due to the ring-breathing vibration. Shirotake,²⁶ for example, assigned another band, at 978 cm^{-1} , to the ring-breathing vibration in his IR study of cytosine. Nevertheless, this band probably is due to a ring vibration. For example, in the IR spectra of cytosine-metal complexes, a medium band near 798 cm^{-1} and a medium band near 780 cm^{-1} were observed and both assigned to the CH + ring vibration.²⁶

Another possible assignment for this band is the so-called amide V band, a NH out-of-plane vibration, suggested by Bandekar et al.^{27,28} Two bands at 853 and 805 cm^{-1} observed in the IR spectrum of uracil were assigned by them to the $N_{(3)}H$ and $N_{(1)}H$ out-of-plane vibration, respectively. For cytosine-metal complexes, similar modes were observed between 804 and 815 cm^{-1} .²⁶ There are problems, however, in assigning the 802 cm^{-1} to the amide V band. To begin, uracil has two NH bonds, yet there is only one strong band at 804 cm^{-1} . While adenine has one NH bond, no band corresponding to the amide V band is observed in its SERS spectrum. A conclusive way to determine whether or not the 802 cm^{-1} band is due to the amide V band is to use N-deuterated uracil, in which case the frequency of the amide V band will decrease to near 600 cm^{-1} . If, on the other hand, the strongest band is mainly due to a ring vibration, its frequency will remain almost constant.

The SERS spectra of adenine, cytosine, and uracil obtained in D_2O colloid solution are shown in Figures 6–8. The hydrogens attached to the nitrogen atoms in these compounds easily undergo deuterium exchange in heavy water. Therefore, these SERS spectra correspond to the N-deuterated species of adenine, cytosine, and uracil. In the SERS spectra of N-deuterated adenine, cytosine, and uracil, a strong band is found at 725 , 788 , and 802

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cm⁻¹, respectively. For the N-deuterated uracil, the frequency of the band discussed above is almost the same as that of ordinary uracil, but for N-deuterated adenine and cytosine the frequencies are red shifted. The amount of red shift is smaller than that expected by a purely -N-D vibration. If this red shift was due to the deuteration of nitrogen atoms of the ring, a similar red shift would be expected for N-deuterated uracil, too. Thus, the slight red shift for N-deuterated adenine and cytosine is due to the mass effect of deuteration of the amino group of these compounds. Uracil does not have an amino group; hence the strong band at 804 cm⁻¹ in its SERS spectrum is due purely to a ring vibration, but the strong bands in the SERS spectra of adenine and cytosine at 739 and 801 cm⁻¹, respectively, are due to a ring vibration with some

contribution from the amino group vibration.

This study coupled with previous ones indicates that SERS spectra may yield rather detailed information regarding the disposition and mode of bonding of molecules adsorbed on appropriate metal surfaces.

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Registry No. OABA, 118-92-3; MABA, 99-05-8; glycine, 56-40-6; α -alanine, 56-41-7; β -alanine, 107-95-9; 6-aminocaproic acid, 60-32-2; adenine, 73-24-5; cytosine, 71-30-7; uracil, 66-22-8; N-deuterated adenine, 51581-02-3; N-deuterated cytosine, 41548-40-7; N-deuterated uracil, 20666-60-8.

Picosecond Time Resolved Raman Scattering Study of Hydrogen Abstraction by Triplet Excited Benzoquinone

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Abstract: The excited state photophysics and H atom abstraction reaction of triplet *p*-benzoquinone in mixed H₂O:alcohol solvents are investigated on the ≈ 20 ps time scale with time-resolved resonance Raman spectroscopy. The triplet is present within 20 ps, and its Raman spectrum is fully developed. It appears that vibrational relaxation is complete at this time. There is no H atom abstraction during the vibrational relaxation process under present conditions. Abstraction proceeds from the relaxed triplet on longer time scales, with a clear kinetic isotope in deuterated solvents. The results also demonstrate that spontaneous Raman pump and probe experiments, at high optical flux, can be performed on chemical transients in low concentration.

Picosecond time-resolved electronic absorption experiments¹ are now common and have been recently extended to the femtosecond scale.² These measurements, however, generally provide *kinetic* but not *structural* information about transient chemical species. Time resolved Raman spectroscopy, if feasible, can be a more useful probe, in the sense that the data provide direct (albeit partial) vibrational information, and often distinguish among similar species exhibiting overlapping optical spectra. Transient Raman scattering experiments invariably require electronic resonance enhancement of the scattering cross section. In several cases where extremely large enhancements are present (e.g., hemes, excited singlet *trans*-stilbene, and reduced methyl viologen), transient Raman experiments have been successfully extended from the nanosecond to the picosecond time scale.³⁻⁸ In this paper the picosecond Raman method is used to investigate the mechanism of hydrogen atom abstraction by electronically excited *p*-benzoquinone. In this reactive system, the two transient species observed (triplet benzoquinone and semiquinone radical) exhibit comparatively modest resonance enhancements.⁹

There are several earlier nanosecond Raman studies on H atom abstraction by triplet excited benzoquinone (hereafter Q(T), see

Figure 1).¹⁰⁻¹² Vibrationally relaxed, equilibrated Q(T) is reported to abstract an H atom from pure neutral water slowly ($\sim 10^{-7}$ s) at room temperature.¹³ Q(T) before reaction must be strongly hydrogen bonded. In alcohol:water mixtures the abstraction reaction is accelerated. In pure water, sequential two-photon absorption leads to fast abstraction. We have previously suggested that some abstraction may occur during the (fast) vibrational relaxation following single photon excitation at 416 nm.^{10,11} Thus, it is interesting to directly observe Q(T) and possible reaction products on a shorter time scale.

An understanding of the picosecond Raman data requires comparison with and extension of the earlier nanosecond data. The basic experiment uses single 416-nm pulses to create transient species (Q(T) and semiquinone radical QH) and generate their electronic resonance Raman spectra within the pulse time width. The nanosecond Raman apparatus produces pulses of ≈ 8 ns width, while the picosecond apparatus produces pulses of ≈ 20 ps. These pulse widths define the effective time scales of the experiments.

The picosecond peak flux on the solution is 10^{10} - 10^{11} W/(cm²·s), several orders of magnitude higher than in nanosecond Raman experiments. We shall demonstrate that, at least in this chemical system, the same resonance Raman scattering and photochemical processes occur as at lower fluxes in the nanosecond experiments.

Observations

The nanosecond laser system, picosecond laser system, and detection system have been previously described.^{8,12} In these

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