

# Surface-enhanced Raman measurements and DFT calculations for L-tryptophan of varying pH in silver sol

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The interaction of L-tryptophan (Trp) with silver colloids was investigated at between pH values of 6.11 and 10.6 of the sol. The measurements of surface-enhanced Raman bands of Trp in the colloidal solution indicate the evolution of interaction between the metal particles and the molecules with increasing pH values for the sol. The experimental observations were explained using the estimated atomic charge distribution in the zwitterionic and anionic forms of the residue, obtained by density functional theory calculations. The variation in the ratio of the spectral intensities of the Fermi resonance bands with the pH reflects the effect of the colloidal environment on Trp. The results obtained can be used as a marker for describing the nature of the interaction of silver colloids with the specific terminus of the residue, at varying pH environments. Copyright © 2012 John Wiley & Sons, Ltd.

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**Keywords:** L-tryptophan; surface-enhanced Raman scattering; Fermi resonance; silver nanoparticles; density functional theory

## Introduction

The basic structural units of proteins and peptides are the amino acids. Tryptophan is an indole derivative in nature, which we find in many compounds of biological importance. The L-stereoisomer of tryptophan, L-tryptophan (Trp), is found in structural or enzyme proteins. For example, this essential amino acid is a precursor for serotonin (a neurotransmitter), melatonin (a neurohormone), and niacin.<sup>[1–3]</sup> Reports on the treatment of epilepsy and depression by derivatives of Trp are available in the literature.<sup>[4,5]</sup> The Trp molecule has been widely studied because of its critical role in governing protein chemistry. The molecular structures and conformations of Trp are well documented.<sup>[6]</sup> Trp exists in three different structural forms<sup>[7]</sup> with varying degrees of protonation depending on the pH of the solution (Fig. 1). Below  $pK_a$ ,  $\text{COO}^-$  is protonated and transforms to  $\text{COOH}$ , whereas at the  $pK_b$  value,  $\text{NH}_3^+$  is half-neutralized to  $\text{NH}_2$  by transferring its proton to the indole nitrogen.

The indole chromophore of Trp is sensitive to its microenvironment. In the excited state, the close lying energy states, La and Lb, of the indole responds significantly to environmental conditions.<sup>[8,9]</sup> Thus, quite a few reports are available in the literature to model Trp in various polar and nonpolar solvents.<sup>[10–12]</sup> Studies on the interaction between metal nanoparticles and amino acids provide a basis to understand the structural properties of the admolecule in metal colloids. Several reports are available in the literature, in which the interactions of Trp with silver (Ag) colloids have been discussed using surface-enhanced Raman scattering (SERS) measurements.<sup>[13–17]</sup> In most of the studies, the strong  $\text{COO}^-$  symmetric stretching and C– $\text{COO}^-$  stretching vibrational mode of Trp in the colloidal metal sol indicate that the carboxylate group is at the interaction site with the metal

particles.<sup>[13–16]</sup> However, the passive role of the above-mentioned terminus of Trp in the Ag–Trp interaction was reported in<sup>[17]</sup>. In a recent report, SERS measurement of Trp, along with the molecular model calculation of Trp as a zwitterionic species, showed that Trp molecules interact with the silver surface through both  $\text{COO}^-$  and  $\text{NH}_2$  groups and the pyrrole terminal is farther from the surface than the benzene ring.<sup>[18]</sup> In this article, the authors claim that the most probable conformation of Trp in the Ag colloidal solution can be achieved only after 12 h of stabilization of the metal–ligand complex. However, it is to be noted that the interaction of the admolecule with metal colloids strongly depends on the nature and pH of the sol. In Ref. [14] the possible effect of different ionic forms of Trp in the SERS spectra is briefly mentioned. To the best of our knowledge, a systematic study on the effect of gradual changes in net atomic charge distributions at different terminals of the molecule in a metal–molecule interaction is still missing in the literature.

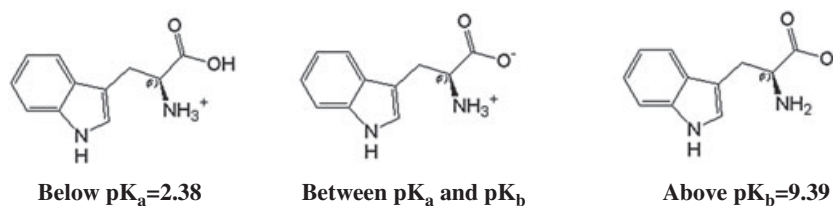
In the present article, we studied the interaction of Trp molecules with Ag colloids of varying pH by optical spectroscopic measurements. The pH is one of the important parameters for monitoring the orientation processes of any molecule at the

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**Figure 1.** Different ionic species of Trp at different pH values.

surface of metal particles. It affects the state of protonation and hence the number and nature of binding sites of the molecules with colloidal particles. For a better understanding and optimization of the interaction activity of the different termini of Trp in an Ag colloidal environment, we mainly focus on the evolution of the plasmon resonance band of the metal particle and also the vibrational bands related to the indole ring, carboxylate group, and amine group of the residue at different pH. In addition, the evolution of the Fermi resonance (FR) band of Trp with the pH of the sol is also discussed. To the best of our knowledge the FR band of Trp in Ag colloidal sol has not been discussed much in the literature.

Section 2 covers the sample preparation technique, which we followed, and other details regarding the instruments that we used for various measurements. Section 3 presents the evolution of (i) the plasmon resonance band of metal particles upon the addition of Trp molecules and (ii) vibrational bands of Trp of varying pH in an Ag colloidal sol. We have carried out density functional theory (DFT) calculations to simulate the expected Raman spectra along with the atomic charge distribution of Trp in zwitterionic and anionic forms. The results obtained from the simulation are available in Section 4. The simulated results describing the characteristics of Trp in different ionic forms were used to understand the pH-dependent interaction of Trp with Ag colloids and is discussed in Section 5. Finally, in Section 6 we summarize our results.

## Materials and methods

Silver nitrate ( $\text{AgNO}_3$ ) and sodium borohydride ( $\text{NaBH}_4$ ) of analytical reagent grades (SRL, India) were used to prepare the Ag sol. A powder sample of L-tryptophan ( $\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_2$ ) was purchased from SRL, India. Ag nanoparticles were prepared by reducing  $\text{AgNO}_3$  in an aqueous solution of  $\text{NaBH}_4$ , following the method reported by Creighton *et al.*<sup>[19]</sup>  $\text{AgNO}_3$  (1 mM, 20 ml) was added to an ice-cold solution of vigorously stirred  $\text{NaBH}_4$  (1 mM, 60 ml) to form Ag colloids. Stirring for 20 min was necessary to stabilize the colloidal solution, which was transparent yellow. When analyzing<sup>[20]</sup> the surface plasmon resonance band of Ag particles, the average size of the particle is estimated to be  $10 \pm 2$  nm. Later, it was left at room temperature for approximately 1 h, allowing the excess  $\text{NaBH}_4$  to evaporate. All measurements were carried out using the sol, which was stabilized overnight.

The SERS is a technique that works best for low concentrations of the probing molecules.<sup>[21]</sup> The intensity of a SERS spectrum also depends on the pH of the sol. The Raman spectra of the aqueous solution of Trp at a concentration below 5 mM could not be measured for all pH. To compare the results (e.g. the enhancement and Raman shift) obtained from SERS and Raman scattering, all measurements were carried out for 5 mM of Trp. A few experiments, focused only on the Raman spectrum of the

Trp molecule, were carried out for 20 mM of Trp, to obtain a good signal-to-noise ratio. The pH of the Ag colloid was 8.50. The pH of the aqueous solution of Trp was adjusted between 3.10 and 11.5 using HCl and NaOH.

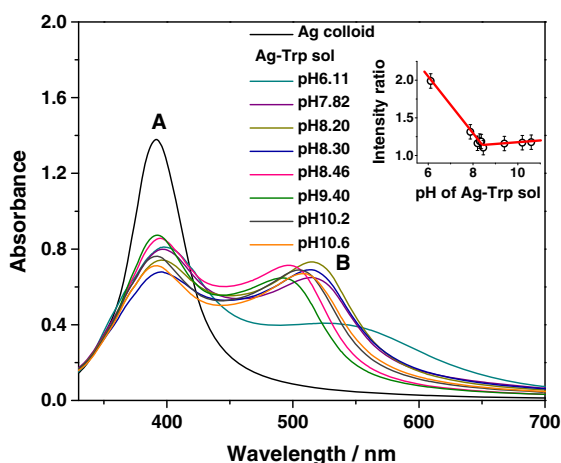
For spectroscopic measurements of Ag–Trp sol, an aqueous solution of Trp (5 mM) was added to the Ag sol in the volume ratio of 1:3. All reported pH values in this article are those of the final Ag–Trp sol. Here, we would like to point out that for pH 3.10 of Trp, the final pH of the Ag–Trp sol was measured to be 6.11. The pH 3.50 of Trp changed the pH of the sol to 7.82. The pH of the final sol, between 6.11 and 7.82, could not be controlled. All measurements were performed at room temperature with a stabilized sol–Trp mixture after 20 min of the adsorption time. We have obtained an identical SERS spectrum of the Ag–Trp sol even after 10 h of adsorption time.

Optical absorption measurements were carried out by a Spectrascan UV 2600. Raman scattering experiments were performed in a  $180^\circ$  scattering geometry using a micro-Raman spectrometer with a 488 nm  $\text{Ar}^+$  laser as an excitation light source. The spectrometer is equipped with an optical microscope (Model BX 41, Olympus, Japan), single monochromator (Model TRIAX550, JY, Horiba, France), an edge filter, and a Peltier-cooled CCD ( $1024 \times 256$  pixel, Model ATE-1024X, JY, Horiba, France) detector. The liquid sample was kept in a cylindrical container (capacity 275  $\mu\text{l}$ ). The incident laser beam was focused inside the container (with sample) forming a cone of base diameter  $\sim 100$   $\mu\text{m}$ . We used a  $10\times$  microscope objective (the objective of the lowest magnification available to us) to get the Raman signal from an appreciable volume of the sol. The input laser power on the sample was fixed at 20 mW to get a good signal-to-noise ratio. The spectra obtained with the lower laser power (not shown here) were of poor signal-to-noise ratios. However, the evolution of the prominent bands of Trp with pH, as obtained from the spectra recorded at the lower laser power, followed a similar trend as observed for the reported conditions. The data acquisition time for each Raman and SERS spectrum was 300 s.

## Results

### Evolution of plasmon resonance band of Ag particles on addition of Trp of different pH

The plasmon resonance spectrum of the Ag colloid is  $\lambda_{\text{max}} = 391$  nm (black line in Fig. 2) because of the dipolar component of the Ag-plasmon resonance in the applied electric field of light. In Fig. 2, the optical absorption spectra over the spectral range between 330 and 700 nm of Ag–Trp recorded at different pH of the sol exhibit two bands, one at around 391 nm (A) because of the surface plasmon resonance of unassociated Ag nanoparticles and another broad band (B) because of the aggregated Ag nanoparticles after the addition of Trp molecules. The change in ratio

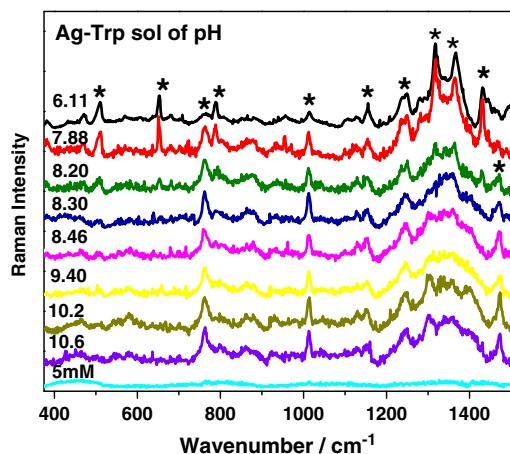


**Figure 2.** Plasmon resonance spectra of Ag colloids at different pH values of Ag-Trp complexes. Inset: ratio of integral intensity of  $I_B/I_A$ .

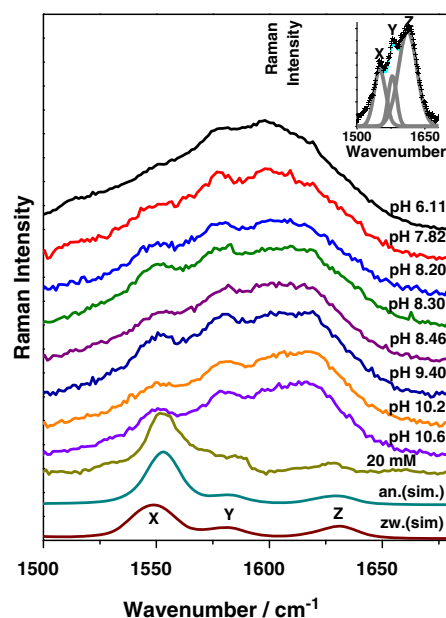
of the integral intensities ( $I_B/I_A$ ) of these two absorption bands with the pH of the Ag-Trp sol is shown in the inset of Fig. 2. The ratio sharply drops between pH 6.11 and 7.82 and then remains nearly constant.

### Evolution of vibrational bands of Trp of different pH in Ag colloidal solution

The SERS spectra of the Trp molecules of different pH in an Ag colloidal environment were recorded over the spectral window between 375 and 1680  $\text{cm}^{-1}$  (Figs 3 and 4). All prominent vibrational bands between 375 and 1500  $\text{cm}^{-1}$  shown by \* marks in Fig. 3 can be assigned to the bond vibration of the molecules (Table S1). The measured spectra between 1500 and 1680  $\text{cm}^{-1}$  are shown separately in Fig. 4. For comparison, the Raman spectrum of the aqueous solution of 5 mM of Trp over the spectral range between 375 and 1500  $\text{cm}^{-1}$  is shown at the bottom of Fig. 3 and, similarly, for 20 mM of Trp over the spectral range between 1500 and 1680  $\text{cm}^{-1}$  is shown by the dark yellow line at the bottom of Fig. 4.



**Figure 3.** Evolution of Raman spectra of Trp over the spectral window between 375 and 1500  $\text{cm}^{-1}$  recorded at different pH values of Ag-Trp sol. Bottom of the figure shows the Raman spectrum of Trp of same concentration in aqueous solution.



**Figure 4.** Evolution of Raman spectra of Trp over the spectral window between 1500 and 1680  $\text{cm}^{-1}$  recorded at different pH values of Ag-Trp sol. Raman spectrum of Trp in aqueous solution is shown by dark yellow line at the bottom of the figure. At the bottom of the figure, magenta and indigo lines correspond to simulated spectra in the zwitterionic and anionic forms of Trp. Inset of the figure shows the Raman spectrum of Trp (in Ag sol) at pH 6.11, over the above spectral range. The + marks are experimental data points. The cyan solid line is the net fitted line after deconvoluting the spectrum, gray lines (will be discussed latter).

### Density functional theory calculations

To understand the evolution of the spectral features of Trp at different pH in a colloidal solution of Ag, we carried out DFT calculations of Trp at different ionic states, reflecting its zwitterionic and anionic forms. Here, we would like to mention that because experimental results in Fig. 2 point towards the electromagnetic interaction of the molecule with aggregated metal colloids, we refrain from carrying out DFT calculations for a complex of Trp and Ag atoms or planes (as we do not have any experimental evidence for chemical bonding between Trp and Ag). It is to be noted that DFT calculations of ligand molecules with metal atoms/planes provide valuable information for SERS via chemisorption (enhancement in Raman bands from the change in polarizability of the admolecule via chemical bond formation with metal atoms). The same formalism does not hold well when describing SERS via electromagnetic enhancement. Electromagnetic enhancement occurs when the molecules are adsorbed at the 'hot sites' (interstitial positions between metal particles), where there is a high local electric field. The orders of magnitude of the enhancement of the electric field at these sites arise because of the interaction of the surface plasmon of metal particles with the incident electromagnetic radiation.<sup>[22,23]</sup> DFT calculations on the basis of the Kohn-Sham<sup>[24]</sup> approach describe the interacting many-particle systems in terms of the noninteracting particle system. The Kohn-Sham Hamiltonian includes the exchange-correlation potential of a homogeneous electron gas density of the system and the potential energy of electrons in the external field of the nuclei. In this formalism, it is nontrivial to solve the Schrödinger equation incorporating the correct interaction



on the indole ring in the zwitterionic and in the anionic states of Trp were (+1.003 unit) and (+0.781 unit), respectively. The charge on the COO<sup>-</sup> terminus changes insignificantly between zwitterionic and anionic states: from -0.997 unit to -0.998 unit. Because of proton transfer,<sup>[29]</sup> the NH<sub>3</sub><sup>+</sup> terminus transforms to NH<sub>2</sub> (Fig. 1), and the electronic charge on this particular terminus changes from +0.550 unit to -0.054 unit.

A careful analysis of the relative variation in intensities of different vibrational bands, as observed in Figs 3 and 4, along with the above results obtained from DFT calculations, reveal an interesting evolution of the metal–molecule interaction with the change in pH of Ag–Trp sol. At the bottom of Fig. 4, we show the simulated Raman spectrum of Trp in both zwitterionic (magenta) and anionic (indigo) forms in an aqueous environment, as obtained from DFT calculations. The spectral features at 1551 (X), 1578 (Y), and 1612 (Z) cm<sup>-1</sup>, correspond to the indole ring stretching and asymmetric bending vibrational modes of the NH<sub>3</sub><sup>+</sup> and COO<sup>-</sup>, respectively, of Trp. As was expected from the DFT calculations, in the measured Raman spectrum (dark yellow line in Fig. 4) of Trp in the aqueous environment, over the spectral range between 1500 and 1680 cm<sup>-1</sup>, the vibrational mode of the indole ring at ~1551 cm<sup>-1</sup> (X) dominates. It appears from Fig. 4 that in the SERS spectra of Trp, the relative intensities of the above spectral lines change appreciably and they appear together as a broad band. To estimate the relative intensity of different Raman bands, each broad spectrum was fitted with three Lorentzian functions (for X, Y, and Z), keeping the peak position, width and intensity of the spectral lines as free-fitting parameters. In the inset of Fig. 4, we show the deconvoluted components (X, Y, and Z) for the spectrum recorded at pH 6.11 by gray lines. The relative intensities of these spectral features appear to be quite different from what we observed for Trp in aqueous solution. The presence of relatively strong vibrational bands Y (1578 cm<sup>-1</sup>) and Z (1612 cm<sup>-1</sup>) of Trp in the Ag–Trp solution in Fig. 4 is a strong evidence of the proximity of the NH<sub>3</sub><sup>+</sup> and COO<sup>-</sup> termini of Trp to the colloidal surface.

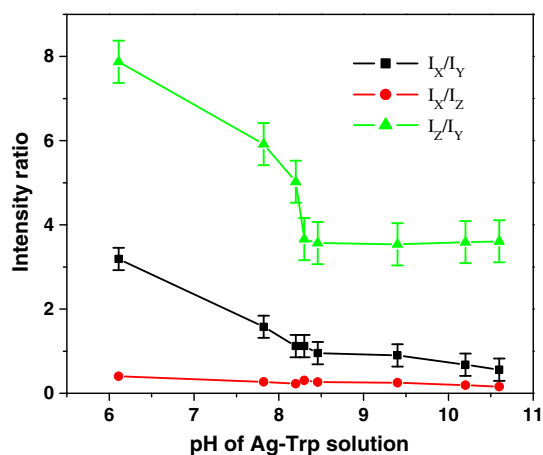
The evolution of the relative intensities of the X, Y, and Z bands with the increase in pH of the Ag–Trp sol are shown in Fig. 7. We will try to explain these observations, using the results obtained from the DFT calculations. Because COO<sup>-</sup> has the highest negative charge, the strongest interaction of this terminus of Trp with Ag<sup>+</sup> colloids results in a most intense asymmetric stretching mode at Z in Fig. 4. The charge on the COO<sup>-</sup> terminus remains unchanged, while the NH<sub>3</sub><sup>+</sup> terminus carries a positive charge in the

zwitterionic state and a negative charge in the anionic state of Trp (with NH<sub>2</sub>). Thus, the interaction between the NH<sub>2</sub> terminus with the Ag<sup>+</sup> colloid becomes stronger with the increase in pH, whereas the interaction between COO<sup>-</sup> and the metal colloid remains unaffected. Thus, the ratio of the intensity of two vibrational modes arising from these two side-ends,  $I_Z/I_Y$ , decreases between the pH values of 6.11 and 8.30 of the Ag–Trp sol, and then remains constant with pH above the value of pK<sub>b</sub> = 9.39 (green symbols in Fig. 7). Similar changes in the spectral profiles from changes in the charge distribution at different termini of the molecule are reflected in the intensity ratio of the vibrational modes of the ring and NH<sub>3</sub><sup>+</sup> terminus  $I_X/I_Y$  (black symbols in Fig. 7). The unchanged ratio of the Raman intensity from COO<sup>-</sup> and the ring,  $I_Z/I_X$  (red symbols in Fig. 7), can be explained by the unaffected effective charge on these two sites of Trp in zwitterionic and anionic forms.

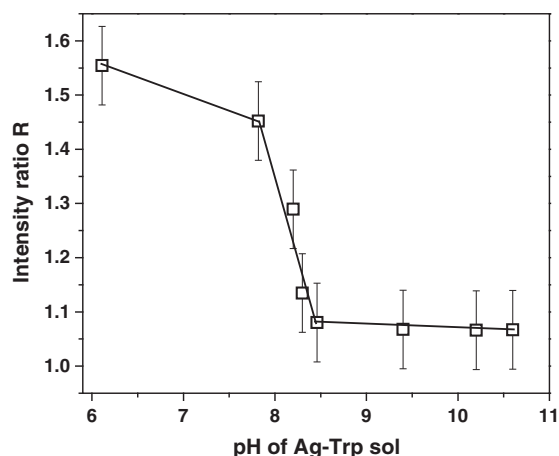
The variation in the relative intensities and Raman shift of other vibrational modes, arising from the indole ring, COO<sup>-</sup> and NH<sub>3</sub><sup>+</sup> termini, in Fig. 3, can also be explained by the above arguments based on the change in the atomic charge distributions at different termini of Trp in two ionic states. For example, the SERS spectral bands at 762 and 1013 cm<sup>-1</sup> of the indole ring do not show a spectral shift or change in intensity with the increase in pH of Ag–Trp sol – signifying a nearly unchanged interaction of the ring with Ag colloids with the increase in pH of the sol. The 1395 cm<sup>-1</sup> band, corresponding to symmetric stretching vibrational modes of COO<sup>-</sup>, sustains with the increase in pH of the sol.

Furthermore, we look into the spectral range between 1300 and 1400 cm<sup>-1</sup> in Fig. 3 in more detail. Within this spectral window, two vibrational bands at 1342 (C) and 1360 cm<sup>-1</sup> (D) are known to appear as a Fermi doublet because of the FR between the fundamental vibration of the indole ring and the combination bands of 920 and 420 cm<sup>-1</sup> and 745 and 600 cm<sup>-1</sup> of Trp.<sup>[30]</sup> The intensity of a combination band is always weak in comparison with that of the fundamental. However, when the frequency of a combination band falls close to that of a fundamental of the same symmetry species, FR occurs. This results in a sharing of intensities between two modes (fundamental and overtones) and a frequency shift in both.<sup>[31]</sup>

For a correct estimation of the intensities of the observed FR bands, we have deconvoluted the spectra, recorded at all pH, over the spectral window between 1190 and 1450 cm<sup>-1</sup>. The deconvoluted spectra for three characteristic pH values are reported in Fig. S1. From the measured integral intensities of the observed bands C and D, the intensity ratio  $R = I_D/I_C$  is plotted for all pH of Ag–Trp sol in Fig. 8. The ratio describes the evolution of the interaction strength of the metal colloid with the molecule. The overlap of frequencies of two combination bands, ( $\nu_{\text{comb1}}$ ,  $\nu_{\text{comb2}}$ ), with a fundamental vibrational frequency is responsible for FR.<sup>[30]</sup>  $\nu_{\text{comb1}} = \nu_1 + \nu_2$  and  $\nu_{\text{comb2}} = \nu_3 + \nu_4$ , where,  $\nu_1 \sim 920$  cm<sup>-1</sup>,  $\nu_2 \sim 420$  cm<sup>-1</sup>,  $\nu_3 \sim 745$  cm<sup>-1</sup> and  $\nu_4 \sim 600$  cm<sup>-1</sup> are related to the out-of-plane vibration of the indole ring. If we assume that the intensities of FR bands arise only from the fundamental, the change in  $R$  with the pH of the sol indicates a relatively stronger/weaker resonance of one of the combination bands (either  $\nu_{\text{comb1}}$  or  $\nu_{\text{comb2}}$ ) or both with the fundamental at the higher pH of the sol. One of the components of the combination band  $\nu_{\text{comb1}}$ ,  $\nu_1$  at 920 cm<sup>-1</sup>, appears because of the N–H deformation of the indole ring and is reported to be sensitive to environmental conditions.<sup>[13]</sup> Thus, in the colloidal environment, the combination band  $\nu_{\text{comb1}}$ , most likely is affected more than  $\nu_{\text{comb2}}$  by the increase in pH of Trp. Also, it is to be noted



**Figure 7.** Intensity ( $I$ ) ratios of the spectral features X, Y, and Z.



**Figure 8.** Change in  $R$  with pH of Trp in Ag-Trp sol. The solid line is the guide to the eye.

that in the SERS spectra for the FR band, the higher band of the Fermi doublet shows a red shift by  $5\text{--}7\text{ cm}^{-1}$ , when the system transforms from a zwitterionic to an anionic state (Table S2), indicating that the combination band(s) are affected by metal-molecule interactions. The above arguments possibly explain the reason for the decrease in  $R$  when the system undergoes the transition from zwitterionic to anionic state, as observed in Fig. 8. With a further increase in the pH of the sol (beyond its pH of 8.5), the residue remains in the anionic state without a further change in charge distribution at its different termini. Hence, the ratio  $R$  remains constant for the higher pH of the sol. Here, we would like to mention that the relative intensities of some of the observed SERS bands are quite different from the simulated Raman intensities in aqueous environment (refer to Figs 3, 5 and Table S2). It explains relatively more intensely observed SERS lines for the bonds of the residue, which are close to the Ag surface.

Spectral interpretation, along with optimization of the structure from DFT calculations provide an insight into the interaction of different termini of Trp with Ag colloids at varying pH. The evolution of the Raman bands of Trp in Ag colloidal sol can be used as markers to determine the strength of interaction and orientation between the two for varying pH. To the best of our knowledge, it is the first report elucidating the correlation between the intensity ratio of the doublet peaks of FR bands over a wide range of pH in a colloidal environment.

## Summary

In this article, we focused on the interaction of different ionic states of Trp with Ag colloids using the evolution of surface-enhanced Raman bands of the residue. DFT calculations for Trp molecules in aqueous environment have been carried out to estimate the atomic charge distributions at different termini of the molecule in zwitterionic and anionic states. The Raman intensities of different vibrational bands of Trp molecules in two ionic states have been simulated. Assuming the electrostatic interaction between different termini of the adsorbates with  $\text{Ag}^+$  particles, the observed evolution of the interaction between Ag colloids and the Trp molecules at varying pH could be explained with the help of the above-simulated results. A possible reason

for a nonmonotonous change in the ratio of the FR band with the pH of the sol has also been discussed.

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## Supporting Information

Supporting information may be found in the online version of this article.

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