

Original Research Article

Integrating computational analysis with IR, Raman, and visible spectroscopy for ampicillin characterization

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Abstract

Purpose: To study the structural, vibrational and electronic properties of ampicillin using a computational approach supported by measurements.

Methods: The structural optimization and vibrational frequencies for density functional theory (DFT; B3LYP/6-311++G (d,p)) calculations were utilized. Calculated frequencies were compared with measured FT-IR and FT-Raman vibrational spectra, while molecular electrostatic potential (MEP) mapping and highest occupied molecular orbit-lowest unoccupied molecular orbit (HOMO-LUMO) modeling were used to characterize the charge distribution in the compound to evaluate chemical reactivity. Finally, quantum chemical modeling was used to characterize the interaction of the ampicillin molecule on silver colloids and predictions from the DFT calculations were then verified using surface-enhanced Raman scattering (SERS) from a hydroxylamine-reduced silver colloid at 532 nm.

Results: The calculated vibrational wavenumbers corresponded closely with FT-IR and FT-Raman data, confirming a valid computational model. The MEP clearly delineated electropositive regions near -NH₃ group and water molecules, which may serve as sites of reactivity in monomer and dimer ampicillin forms. The HOMO-LUMO models clarified the electronic transitional nature of the compound while DFT modeling on ampicillin adsorption showed good agreement with an effective interaction with silver colloids using SERS.

Conclusion: This study demonstrates that DFT and spectroscopic studies accurately characterize the structural, vibrational and electronic properties of ampicillin, as well as an interaction with silver nanoparticles that supports its use as a diagnostic agent in surface-enhanced spectroscopy.

Keywords: Ampicillin, Computational analysis, HOMO-LUMO, FT-IR, Surface-enhanced Raman scattering (SERS)

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INTRODUCTION

β -Lactam antibiotics, which include penicillins, cephalosporins and carbapenems, are still important in the treatment of bacterial infections, yet resistance through β -lactamase production,

altered penicillin-binding proteins, decreased outer-membrane permeability and efflux has been observed in an increasing number of Gram-positive or Gram-negative organisms [1]. One alternative approach to overcome resistance attributed to permeability is the use of microbial

iron-uptake Trojan horse pathways. Siderophores are low molecular weight iron chelators that are recognized by outer-membrane receptors. They enhance antibiotic permeability via conjugation of the antibiotic to the siderophore to actively transport the antibiotic into the bacterial cell [2]. Reports have demonstrated that siderophores enhance β -lactam activity, exemplified by enhanced ampicillin and amoxicillin activities against resistant Gram-negative strains, including *Pseudomonas aeruginosa* [3]. Moreover, the reports have also inspired the development of siderophore-based monobactams with animal model activity [4]. Previous base-based investigations have mainly utilized catecholate- and hydroxamate-based siderophores [5]. However, the varieties of naturally occurring acid tetramics as iron chelators have not been fully utilized, despite their potential antibacterial activity [6]. Ampicillin is a semisynthetic penicillin with broad activity against pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Escherichia coli*, and it is the prototypical β -lactam with excellent oral absorption and clinical use. However, an in-depth understanding of its structural reactivity, intermolecular interactions and overall behavior at biointerfaces remains poorly understood. Recent advancements in computational chemistry, especially shown by Density Functional Theory (DFT) calculations, enable a more detailed description of electronic structure [7], vibrational spectra and adsorption on surfaces [8]. These methods can be complemented by experimental readings, such as FT-IR, FT-Raman, and surface-enhanced Raman spectroscopy (SERS), creating powerful methods to study molecular geometry, vibrational assignments and electron density distributions in frontier molecular orbitals. Researchers often use B3LYP functional with 6-311++G(d,p) basis set to accurately predict structural parameters for β -lactams [9]. The use of SERS, by measuring localized surface plasmon resonance of Ag nanoparticles, also allows visualization of adsorption geometries that could be applied across multiple applications, such as the design of nanosensors and drug delivery systems, as well as environmental detection [10].

In this study, experimental spectroscopy was coupled with DFT-based modeling to characterize and optimize ampicillin's molecular structure and assign its vibrational modes, validate computational predictions with FT-IR and FT-Raman experimental readings, analyze molecular electronic properties using Molecular Electrostatic Potential and HOMO–LUMO analysis, and explore SERS and DFT modeling

of adsorption behavior on silver colloidal surfaces. The combined perspective will lead to improved understanding of ampicillin as a molecular entity and cross-cutting intramolecular and intermolecular interactions with implications for use in the pharmaceutical field, for diagnosing purposes and in nanotechnology applications [11].

EXPERIMENTAL

Instrumental analysis

The FTIR spectrum of ampicillin powder (2 g; Molecular Weight: 371.39; DL number: MFCD00064313; UNSPSC Code: 12352107; PubChem Substance ID: 24891463; Hi-Media) was captured at 25 °C using a standard (Equinox 55 Model) FTIR spectrometer (25 FT-IR Buckner) outfitted with a detector ((470-442500) Thermo Nicolet IS50 DTGS detector) and a sampling setup (ATR) with a Zinc selenium crystal (630). Backscattering geometry was used to record the FT-Raman spectrum at a spectral range of 3600 - 50 cm^{-1} using a Germanium detector (Bruker) Raman accessory and a cold nitrogen detector. The strength of the laser was measured to be 300 mW at the site of the sample and by combining all 32 scans, a resolution of 4 cm^{-1} for all spectra was obtained. An Nd: YAG, 532 nm illuminating doubled frequency laser, and a DeltaNu Advantage spectrometer (DeltaNu, Laramie, WY) were used to record the 10^{-4} M ampicillin SERS-derived spectra. The resolution of the spectral was 10 cm^{-1} , with the laser energy at 41 mW.

Chemical reactions

Silver colloidal SERS base was produced by the reduction of Ag^+ with HNO_3 (Nitric Acid) [12]. The AgNO_3 (0.015 g) was mixed with 91 mL of double-distilled water. In a different receiver, $\text{HONH}_2\cdot\text{HCl}$ (0.017 g) dissolved in 10.0 mL of distilled water was mixed with 0.250 mL of a 2 mol/L (v) solution of sodium hydroxide. The AgNO_3 solution was mixed with hydroxylamine/sodium hydroxide solution. A colloidal grey-brown solution was produced and then agitated for 10 minutes. The pH of silver colloid was 8.5 when determined immediately after preparation. All compounds were of analytical grade.

Computational details

To calculate the molecular electrostatic potential, molecular geometry optimization and oscillational spectra using the Gaussian 03W software package [13], the density-functional-theory (DFT)

methods with B3LYP and the conventional 6-31G(d) basis set were used. During geometry optimization, there was no symmetry limitation. Non-fictitious and vibrational frequencies were created and calculated at optimal geometries to confirm a local minimum correlation on the potential-energy surface. The assigned frequency was supported by a correlation between experimental and theoretically estimated vibrational frequencies, which constructed theoretical frequencies and the Raman spectra intensity pattern. The Raman calculated intensities (S_i) were changed to comparative intensities (I_i) using Eq 1 [14].

$$I_i = \frac{f(v_o - v_i)^4 S_i}{v_i \left[1 - \exp\left(-\frac{hc v_i}{kT}\right) \right]} \dots\dots\dots (1)$$

The wavenumber (excited) is v_o , v_i = wave number of the i^{th} mode, C = speed of light, h and k = Planck's and Boltzmann's constants, T = temperature. The vibrational movements that are mainly responsible for various normal modes were set according to B3LYP method combined with 6-31G(d) basis. Also, according to Scott and Radom's suggestion, 0.9614 was determined to have scaled the computed wavenumbers [15]. Direct comparisons of predicted and experimental spectra, taking into consideration both the intensity pattern and frequency, as well as comparisons with the vibrational spectra of related chemicals [16], were used to assist with mode assignment.

Statistical analysis

The molecular orbital compositions were obtained by Gauss View 3.0. A brief and reproducible statistical analysis section is included here, which can easily be inserted (or modified) in another study, along with the agreement statistics from such tables of experimental and DFT-predicted vibrational wavenumbers.

FT-IR (FT-IR vs B3LYP IR)

$n = 29$ matched modes, MAE = 2.59 cm^{-1} , RMSE = 2.77 cm^{-1} , Mean signed error = -0.72 cm^{-1} , (theoretical values were on average 0.72 cm^{-1} lower than experiment, SD of signed differences = 2.72 cm^{-1} , Linear regression: slope = 0.9999 , intercept = -0.525 cm^{-1} , Pearson $r = 0.999997$, $R^2 = 0.999993$

Raman (FT-Raman vs B3LYP Raman)

$n = 29$ matched modes, MAE = 4.00 cm^{-1} , RMSE = 4.51 cm^{-1} , mean signed error = -0.76 cm^{-1}

(theoretical values were on average 0.76 cm^{-1} lower than experiment, SD of signed differences = 4.52 cm^{-1} , Linear regression: slope = 1.0030 , intercept = -3.574 cm^{-1} , Pearson $r = 0.999956$, $R^2 = 0.999911$.

The DFT/B3LYP predictions align very closely to experiment: both FT-IR and FT-Raman correlations yield R^2 values of > 0.9999 (IR) and ~ 0.9999 (Raman) and slopes of ~ 1 (or very close to 1), suggesting there is negligible systematic scaling error over the measured range. Also, MAE and RMSE (IR: ~ 2.6 and 2.8 cm^{-1} ; Raman: ~ 4.0 and 4.5 cm^{-1}) values are small and within what would typically be considered the acceptable deviations associated with harmonic DFT frequencies (after standard scaling), thus lending confidence to the computational model for vibrational assignment. Furthermore, the mean signed errors were also small ($< 1 \text{ cm}^{-1}$), suggesting that there was no systematic over- or under-estimation of wavenumbers by the computational method. The somewhat larger MAE/RMSE for Raman suggests either larger deviation in Raman-mode prediction, or perhaps some differences in assignment/peak picking, but overall agreement remains excellent. A statistical comparison between calculated and experimental vibrational wavenumbers was conducted by comparing vibrational mode assignments as provided in Tables 2 – 3. For FT-IR, the 29 matched modes yielded a MAE of 2.59 cm^{-1} and RMSE of 2.77 cm^{-1} ; linear regression of calculated vs experimental wavenumbers gave a slope of 0.9999 and an R^2 of 0.99999 , indicating nearly identical results. For FT-Raman (29 matched modes) MAE = 4.00 cm^{-1} , RMSE = 4.51 cm^{-1} , slope = 1.0030 , and $R^2 = 0.99991$. The mean signed errors were small (-0.7 to -0.8 cm^{-1}), indicating minimal systematic bias. The results confirm that B3LYP/6-311++G(d,p) model simulates the experimental vibrational spectra with reasonable accuracy and supports the vibrational assignments.

RESULTS

Energy and geometry optimization

The crystal molecular structure of ampicillin is known. Therefore, the optimized structures were contrasted with the structural properties of the crystal. Figure 1 shows the structure and atomic numbering system of the compound, while the bond lengths and bond angles of ampicillin are contained in Table 1. One of the key ideas in the evolving density functional theory (DFT)-based models of the electronic framework of atoms and compounds is the electron correlation. Accurately

accounting for electron-electron correlation is the main issue in quantum chemical computations.

Molecular electrostatic potential (MEP)

The vibration frequencies of ampicillin were determined after geometry optimization.

Optimized geometry, shown in Figure 1, did not provide any imaginary frequencies, thus indicating a potential-energy surface minimum. Furthermore, the potential molecular electrostatic map of ampicillin is illustrated in Figure 2.

Table 1: Bond lengths (Å) and bond angles (°) experimental and optimized ampicillin at B3LYP/6-311G (d) of theory level in the gas phase

Bond lengths (Å)	Experimental	Calculated	Bond angle (°)	Experimental	Calculated
S7- C2	1.74	1.78	S6-C2-C7	102.05	103.15
C2-C4	1.45	1.48	C2-C3-N1	104.86	106.09
C3-N1	1.36	1.34	C5-N10-C15	106.86	109.89
N10-C5	1.36	1.36	N1-C4-O9	111.59	115.26
C3-C8	1.42	1.46	C3-C11-O13	117.24	115.18
C3-C7	1.42	1.44	N19-C16-C18	87.44	87.49
C4-N1	1.27	1.39	C2-C5-C4	84.64	82.85
C4-O9	1.09	1.19	C7-C3-C8	127.25	125.64
C7-C12	1.46	1.42	O13-C8-O14	129.10	129.25
C8-O14	1.15	1.29	C3-C4- N1	119.20	119.68
C8-O13	1.09	1.25	C16-N19-C15	119.36	119.75
C16-N19	1.32	1.32	N10-C15-O17	123.78	120.82
N10-C5	1.23	1.26	C8-C3-N1	117.76	118.42
C16-O18	1.19	1.11	O13-C8-O14	102.83	105.42
C20-C22	1.44	1.43	C12-C7-C12	107.67	108.75
C15-N10	1.47	1.45	Bond lengths (Å)	Experimental	Calculated
C2-C5	1.41	1.42			
C2-H22	0.94	0.91			
			C11-H20	1.16	1.09
			C12-H21	0.87	0.97

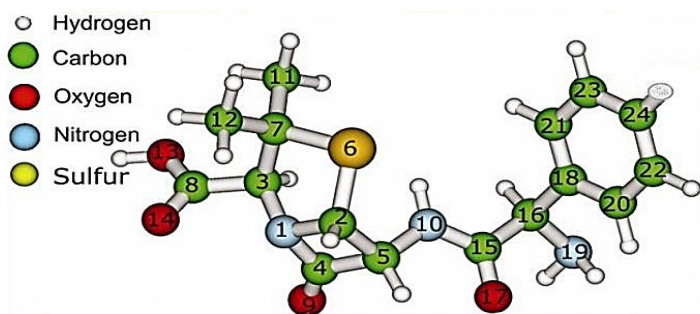


Figure 1: Optimized molecular structure of Ampicillin

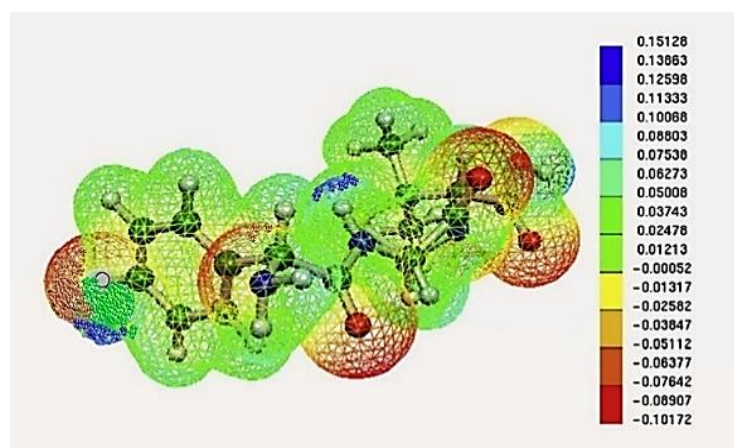


Figure 2: Calculated potential molecular electrostatic map of ampicillin (atomic units)

Ampicillin FTIR spectrum

Observed and predicted ampicillin FTIR spectra are displayed in Figure 3. The results were derived from significant alterations in the dipole moment of the molecule caused by the vibration mode. Hence, the CaO stretching vibrations for ampicillin exhibited strong IR absorptions. According to theoretical DFT calculations, the stretching vibrations of C₁₅-O₁₇ and C₈-O₁₄, respectively, were responsible for the 1685 and 1773 cm⁻¹ bands. Table 2 displays experimental FTIR assignments and estimated ampicillin bands. Other strong IR bands, as seen in Figure 4, include those at 1485 cm⁻¹, which were largely associated with N₁₀-C₁₅ stretching and N₁₀H bending, and 1125 cm⁻¹, primarily associated with O₁₃-C₈ stretching and C₈-O₁₄-O₁₃-H bending vibration.

Raman and SERS spectra of Ampicillin

The ampicillin FT-Raman, estimated Raman DFT and SERS spectra are displayed in Figure 5. Strong fluorescence emission obscures ampicillin Raman spectra acquired with the 535 nm laser line are demonstrated in Figure 5, along with FT-

Raman spectrum obtained with a 1057 nm laser. There was a strong comparison between the theoretical and experimental FT-Raman signals. In Table 3, the appointment of the experimental FT-Raman, DFT computed Raman and SERS bands of ampicillin are listed. Experimental band data were visually linked with the estimated appointment of the FT-Raman and SERS spectra by accounting for wave number and intensity. The aromatic ring symmetric vibration appeared as strong Raman spectra intensity spectrum. The symmetric vibration was found in the estimated Raman spectrum of ampicillin at 864 cm⁻¹; and it displayed as a strong band at 849 cm⁻¹ in the FT-Raman spectrum of ampicillin. Other ampicillin FT-Raman strong bands identified in the spectrum related to the benzene ring were visible at 1620, 1255 and 1175 cm⁻¹, respectively. The aforementioned results are linked to vibrations of the C-C stretching and CAH bending in the aromatic ring, respectively (Table 3).

HOMO-LUMO energy gap

The excitation energies can be calculated in a variety of ways.

Table 2: Experimental and calculated frequencies of ampicillin with band assignment

Experimental wave numbers (cm ⁻¹) FTIR/ATR	Calculated wave numbers (cm ⁻¹) B3LYP	Band assignment
655	661	δ(O ₉ -H)
731	741	δ(C ₄ H-O ₉ -H) ν(C ₇ C ₁₂)
831	819	d(CH- ² ring)
845	841	Bending CH ² ring
871	862	² Ring δ(CH ² ring) + δ(N ₁₀ -H ₅)
919	912	ν(C ₁₆ -C ₁₅ -H ₃ C ₅ -C ₂) + δ(N ₁ -H ₇)
937	937	ν(C ₃ -C ₈ -H, C ₁₂ -C ₁₁) + δ(O ₁₄ -H) + δ(C ₁₁ -H ₇)
956	952	m(NH ₂ -C ₁₆)
983	972	bending CH ² ring
1019	1043	ν(N ₁ -C ₃ , N ₁ -C ₄) + δ(N ₁₀ -H ₈)
1117	1107	ν(O ₁₇ -C ₁₅) + δ(C ₈ -O ₁ -H) + δ(O ₁₄ -H)
1161	1157	δ(CH ² Ring) + δ(O ₁₇ -H)
1173	1172	d(N ₁₀ -H ₉ , N ₁₀ -H)
1215	1205	ν(C ₁₅ -N ₁₀ , C ₄ -N ₁)
1281	1275	ν(C ₄ -N ₁) + δ(N ₁₀ -H)
1325	1329	ν(C-C ² Ring)
1375	1367	δ(C ₃ -C ₈ -O ₁₄ -H) + ν(C ₂₁ -C ₂₃)
1447	1429	ν(C-C ² Ring) + δ(O ₉ -H)
1477	1487	ν(N ₁₀ -C ₅) + δ(N ₁ -H)
1573	1579	δ(C-H ² Ring) + δ(O ₁₀ -H)
1681	1678	m(C ₁₆ -O ₁₉)
1771	1787	ν(C ₃ -N ₁) + δ(C ₇ -H)
2964	2957	ν(C ₁₁ -H)
3031	3041	vas(C ₂₃ -H, C ₂₂ -H)
3365	3377	vas(N ₁₀ -H)
3447	3457	ν(N ₁₄ -H)
3525	3530	ν(O ₁₄ -H)

m: stretch; mas: asym stretch; d: bending; q: rocking; op: out of plane; ring¹: thiazole ring (N₁-C₂-S₆-C₇-C₃); ring²: benzene ring

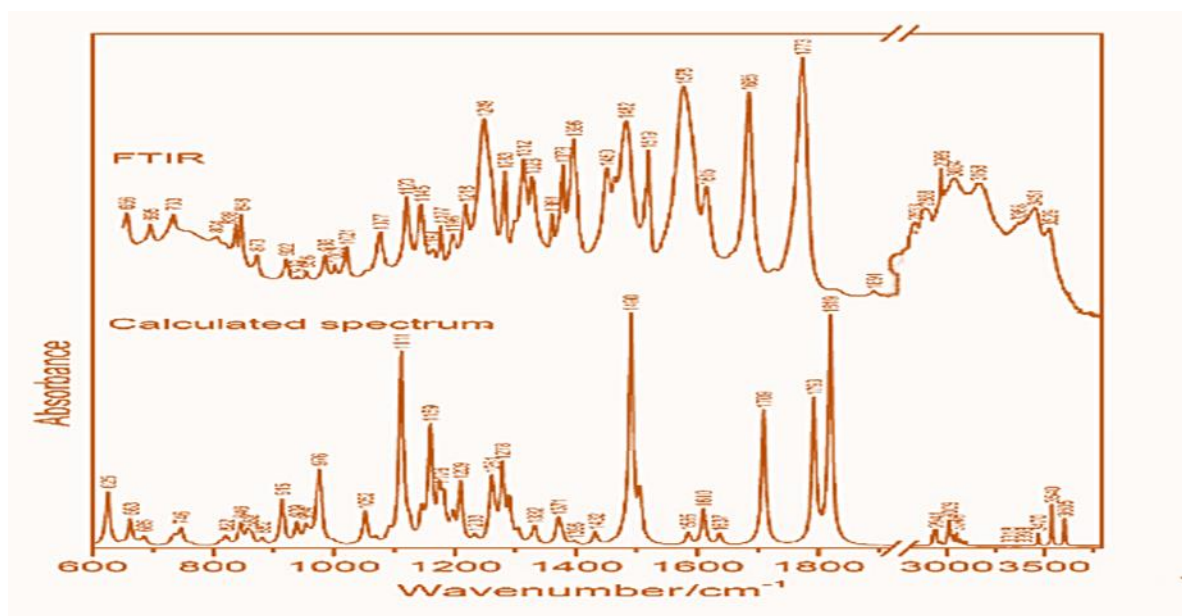


Figure 3: FT-IR spectra of ampicillin (experimental vs calculated)

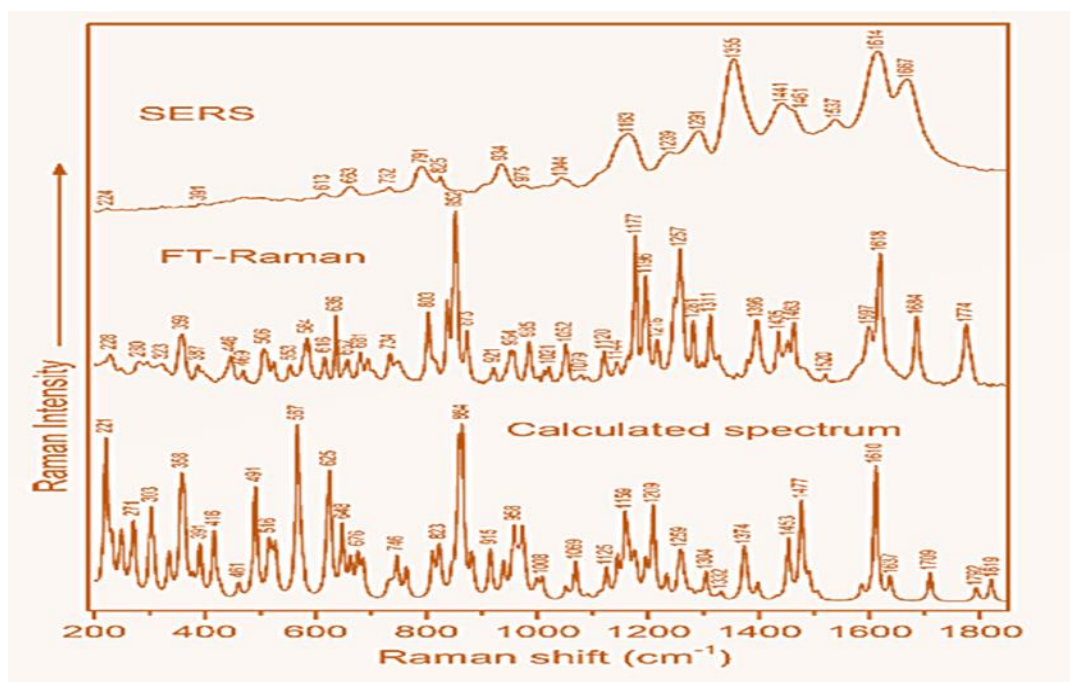
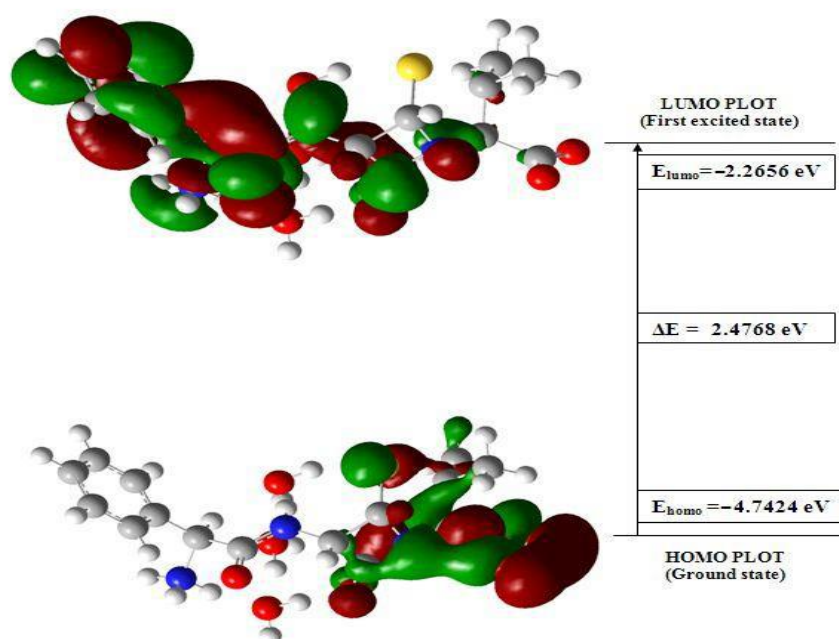


Table 3: SERS, Raman and calculated Raman Oscillational frequencies of ampicillin

Exp. wave numbers (cm ⁻¹)	Calculated wave numbers (cm ⁻¹)				Band assignment
SERS	FT-Raman	B ₃ LYP	FT-IR	B ₃ LYP	
400	228	225	656	659	$\rho(\text{NH}_2)$
410	280	278	733	735	$\rho(\text{CH}_3 - \text{NH}_2) + \text{op. } ^2\text{ring def.}$
440	323	319	834	832	$\rho(\text{CH}_3) + \delta(\text{CH}_3)$
490	387	391	873	870	op. ² ring, ¹ ring def + op. ² ring, ¹ ring bend.
510	446	439	922	919	ip. ¹ ring def + $\delta(\text{COH})$
530	469	466	939	939	ip. ¹ ring def. + $\delta(\text{NH}) + \delta(\text{CH}_3)$
530	525	519	956	954	op. ² ring bend + $\delta(\text{NH})$
550	553	549	986	982	op. ² ring, ¹ ring def + op. ² ring, ¹ ring ben
600	584	579	1021	1025	$m(\text{C}_7\text{S}_6) + \delta(\text{C}_7\text{CH}_3)$
611	607	618	1120	1118	$\delta(\text{O}_{13}\text{H}) + \delta(\text{CH}_3)$
661	659	651	1164	1161	ip. ² ring def.
821	829	827	1249	1247	op. bending ² ring + d(CH. ² ring)
890	852	856	1283	1280	$\delta(\text{N}_{19}\text{H}_2)$
929	919	920	1328	1331	$v(\text{C}_{15}-\text{C}_{16}\text{H}, \text{C}_2\text{C}_5), v(\text{C}_7-\text{C}_3, \text{C}_3-\text{C}_8)$
950	954	957	1378	1375	$\rho(\text{CH}_3) + \text{op. bending CH ring}_2$
990	985	982	1396	1398	$\delta(\text{CH}) + v(\text{C}_{16}\text{NH}_2) + \delta(\text{C}_{15}\text{C}_{16}\text{NH}_2)$
1001	1021	1018	1450	1449	$\rho(\text{CH}_3)$
1041	1052	1050	1482	1485	$v(\text{C}_{16}\text{NH}_2) + \delta(\text{NH}_2) + \delta(\text{CH})$
1090	1120	1121	1578	1581	$\delta(\text{C}_{12}\text{C}_7\text{C}_{11}) + \rho(\text{CH}_3)$
1159	1177	1172	1615	1610	$\delta(\text{O}_{25}-\text{H}) + \delta(\text{N}_{19}-\text{H}_2)$
1179	1196	1201	1685	1681	$v(\text{C}_5-\text{N}_{10}) + \delta(\text{N}_{10}-\text{H})$
1237	1253	1249	1773	1775	$v(\text{C}_{24}\text{O}-\text{H}) + d(\text{C}-\text{H } ^2\text{ring})$
1289	1310	1315	2969	2966	$\delta(\text{N}-\text{H}) + \delta(\text{C}-\text{H } ^2\text{ring})$
1352	1372	1370	3034	3036	$\tau(\text{N}_{19}-\text{H}_2) + \delta(\text{C}_{16}-\text{H})$
1457	1455	1459	3451	3449	$\delta_{\text{as}}(\text{CH}_3)$
1611	1609	1605	3529	3531	$v(\text{C}-\text{C } ^2\text{ring}) + \delta(\text{N}_{19}-\text{H})$
1665	1651	1649	3677	3678	$\delta(\text{N}_{19}-\text{H})$
1700	1697	1701	3755	3752	$\delta(\text{N}_{10}-\text{H}) + v(\text{C}_{15}-\text{O}_{17})$
1710	1774	1779	3822	3819	$v(\text{C}_8-\text{O}_{14}) + \delta(\text{C}_3-\text{H})$

v: Stretching; ρ : rocking; τ : twisting; δ : bending; ip: In plane; op: out of plane; ring 1: thiazole ring (N₁-C₂-S₆-C₇-C₃); ring²: benzene ring (C₁₈-C₂₀-C₂₁-C₂₂-C₂₃-C₂₄)

**Figure 5:** HOMO-LUMO plot of ampicillin with orbitals involved in electronic transitions in gas phase

Ampicillin adsorption geometry on surface of silver

The normal modes are improved by changes in perpendicular polarizability to the surface while implementing the SERS surface-selection principles [17]. Theoretically, lone pair electrons on the O, N, and S atoms and the p electrons of the rings can be used to determine how ampicillin interacts with the surface of silver. The ampicillin adsorption geometry on colloidal silver particles was calculated and is shown in Figure 6. Its absorption to the surface of silver was inferred due to the electronic potential map and various marker signals. As demonstrated in Figure 6, the oxygen atoms and the N₁₇H amino group had the highest electron densities, thus allowing the molecule to interact with the silver surface through them. The SERS spectra show a very weak version of the powerful FT-Raman signal at 855 cm⁻¹, which was attributed to the ring structure of the benzene molecule. Additionally, the bands caused by CAH stretching vibrations were absent from the greater SERS spectrum wavenumber range. In light of these factors, the benzene ring seemed to exhibit a dominant parallel coordination to the silver surface. Because of a twisting vibration, the N₁₇H amino band (1357 cm⁻¹) in the SERS spectrum had particularly great intensity, indicating that this group was close to the silver surface [11]. As a result of N₁₇H deformation vibrations, strong bands in the SERS spectra were also seen at 1617 and 1665 cm⁻¹ (Table 3), alluding to the ampicillin molecule's adsorption to the silver surface via N₁₇ atom.

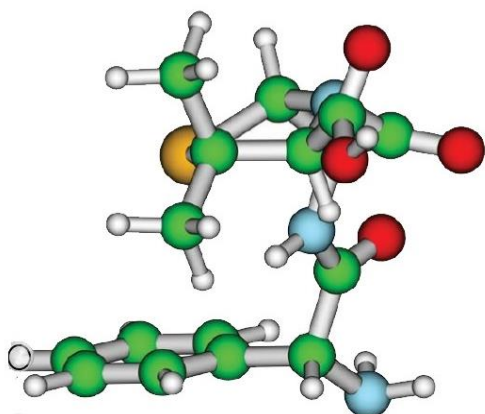


Figure 6: Adsorption geometry in Schematic view of ampicillin on silver surface

DISCUSSION

Findings from this investigation showed a robust relationship between experimental evidence and theoretical expectations in the characterization of

ampicillin. The B3LYP/6-311G(d) level of theory geometry optimization produced bond lengths and angles that nearly matched experimental values, demonstrating the validity of the DFT method. Both -NH₂ group and electronegative oxygen atoms were recognized as important reactive sites by the Molecular Electrostatic Potential (MEP) map, indicating their roles in intermolecular interactions. Excellent agreement between calculated and observed FT-IR and FT-Raman spectra was shown by vibrational mode analysis, especially in bands that corresponded to C=O and N-H stretching. In addition, good chemical stability and mild electronic reactivity were indicated by a HOMO-LUMO energy gap of 2.4768 eV. Ampicillin firmly adsorbs onto silver nanoparticles via nitrogen and oxygen atoms, greatly increasing Raman signals and producing distinctive vibrational changes, according to Surface-Enhanced Raman Spectroscopy (SERS).

The adsorption geometry was further validated by the parallel alignment of benzene ring on the silver surface and the suppression of aromatic C-H stretching bands. These results offer a thorough comprehension of the surface, electrical, and structural interaction characteristics of ampicillin, with implications for molecular diagnostics, drug creation, and sensing applications. By altering the molecule's vibrational modes upon engagement with the metallic surface, ampicillin's adsorption onto silver nanoparticles (AgNPs) is essential for increasing SERS signal intensities. According to the current study, ampicillin preferentially attaches itself to AgNPs through electronegative oxygen and nitrogen atoms, especially those from the amino (-NH₂) and carbonyl (-C=O) functional groups. Molecular Electrostatic Potential (MEP) mapping, which emphasizes the high electron density close to these reactive sites and promotes strong interactions with silver, lends additional evidence to this binding process [18]. Furthermore, π -electron interactions contribute to surface binding, but with a lesser enhancement than perpendicular adsorption, as indicated by the benzene ring's parallel alignment with the silver surface, which was deduced using SERS selection rules. Certain molecule orientations may result in reduced Raman scattering because of decreased perpendicular polarizability with respect to the surface, as evidenced by the suppression of particular vibrational bands, such as the aromatic C-H stretching modes [19].

Two main mechanisms are responsible for the notable boost in ampicillin's SERS spectra: When excited by a 532 nm laser, the AgNPs

produce a significant localized surface plasmon resonance (LSPR) effect that increases the local electromagnetic field surrounding adsorbed molecules. Significant improvement is seen in vibrational modes including N-H and C=O stretching vibrations whose dipole moment change is perpendicular to the surface. The functional groups of ampicillins and the silver surface undergo direct charge transfer, which modifies the electron density distribution and raises Raman cross-sections for particular vibrational transitions. The HOMO-LUMO energy gap (2.4768 eV) indicates that electronic transitions enhance signal intensities by facilitating charge transfer interactions.

Significant ramifications for pharmaceutical and therapeutic applications result from the understanding of ampicillin's adsorption behavior and how it affects SERS intensities: Trace-level identification of ampicillin in medication formulations is made possible by SERS-based detection, which guarantees quality assurance and the identification of counterfeits. Differentiating ampicillin from other β -lactam antibiotics is made possible by its distinct spectral fingerprint. To optimize dosage and stop antimicrobial resistance, SERS can be used to monitor antibiotic concentrations in biological fluids (such as blood plasma and urine) in real time. Using ampicillin's potent adsorption characteristic on AgNPs, extremely selective biosensors can be created. SERS-based platforms improve contamination surveillance by detecting ampicillin residues in food, goods and water sources. In complicated matrices, functionalized AgNPs with adjustable adsorption capabilities can be created for targeted antibiotic detection. In order to thoroughly analyze ampicillin, this study combines computational and experimental methods, offering insights into its molecular structure, vibrational characteristics, electrical features, and adsorption behavior. The B3LYP functional and 6-311++G (d, p) basis set were used in Density Functional Theory (DFT) to accurately predict and evaluate the improved molecular geometry and vibrational spectra against experimental findings. The accuracy of computational approaches in predicting molecule vibrational modes is confirmed by the strong agreement between theoretical and experimental FT-IR and FT-Raman spectra. Robustness of the employed theoretical model was demonstrated by the bond lengths and bond angles found in the optimized molecular structure of ampicillin, which closely matched experimental values. Strong absorption bands in FT-IR spectrum were indicative of the amide and β -lactam groups' stretching vibrations, which were also seen in the calculated spectra. In close agreement with DFT

simulations, the C=O stretching vibrations, which are essential for describing β -lactam antibiotics, were detected in FT-IR at 1685 cm^{-1} and 1773 cm^{-1} and the molecular structural findings were further supported by Raman spectra analysis, which demonstrated the important vibrational contributions from aromatic ring stretching and N-H bending vibrations. Important information about ampicillin's electronic structure was revealed by HOMO-LUMO, which showed an energy gap of 2.4768 eV. Ampicillin appears to have stable yet reactive properties, which makes it appropriate for biological interactions, based on this moderate energy gap. The electropositive areas, especially those close to -NH₃ group, are crucial for molecular interactions, according to the Molecular Electrostatic Potential (MEP) mapping, which also suggests possible locations for nucleophilic assaults and hydrogen bonding. These results are in line with the antibiotic's known mode of action, which involves its β -lactam ring interacting with bacterial transpeptidases. SERS was used to investigate ampicillin's adsorption behavior on a silver colloidal surface. A preferential interaction between ampicillin and the silver nanoparticles is suggested by the substantial amplification of Raman signals, especially in areas linked to C-N, C=O, and N-H vibrations. Shifts in vibrational bands confirm the adsorption geometry analysis, which showed that oxygen and nitrogen atoms are important for surface binding. According to the faint SERS signal at 855 cm^{-1} , which is usually strong in Raman spectra, the benzene ring minimizes direct contacts by adopting a parallel orientation with respect to the silver surface. These results are consistent with SERS selection criteria, which favor vibrational modes with high perpendicular polarizability.

This study's combination of spectroscopic and computational methods offers a more thorough comprehension of ampicillin's molecular characteristics. The results show how well DFT-based models predict electrical properties and vibrational spectra, which can be used for structural study of other β -lactam antibiotics. Key adsorption sites identified in SERS research also provide important information for pharmaceutical applications, such as sensor-based antibiotic detection and drug delivery systems. All things considered, this work demonstrates how well quantum chemistry simulations and experimental spectroscopy methods may be combined to better understand molecular behavior, interactions, and reactivity. In addition to aiding in the characterization of antibiotics, these results open up new avenues for research on drug interactions with metal surfaces, which is

important for the development of biosensors and nanomedicine.

CONCLUSION

This study successfully characterizes the molecular structure, vibrational characteristics, electrical behavior, and surface contacts of ampicillin by combining computational and experimental methods. The accuracy of the theoretical models is validated by DFT calculations using B3LYP functional and 6-311++G (d, p) basis set, which produces optimized molecular geometry and vibrational spectra that closely match experimental FT-IR and FT-Raman data. While MEP mapping indicates important locations for molecular interactions, HOMO-LUMO analysis shows a 2.4768 eV energy gap, indicating moderate stability and reactivity. Ampicillin's significant adsorption on silver colloid surfaces was revealed by SERS research. This adsorption is impacted by nitrogen and oxygen atoms, which increase vibrational intensities and shifts. These results demonstrate how computational techniques may accurately anticipate and validate molecular activity, offering valuable information for developing β -lactam antibiotics, enhancing drug formulation, and developing SERS-based antibiotic detection, which ultimately provides insightful information for studies on antimicrobials and nanomedicine.

DECLARATIONS

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Ethical approval

None provided.

Use of Artificial Intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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