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Probing the structure of Moxifloxacin and Norfloxacin by density functional theory and Raman spectroscopy

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In the present work, structures of two fluoroquinolone molecules namely Moxifloxacin and Norfloxacin are investigated using density functional theory and the Raman spectroscopy. The density functional theory calculation with B3LYP-6-31+G(d,p) level of theory reveals several structures of two molecules. The low energy stable structures of Moxifloxacin show multiple intramolecular H-bonds which could be responsible for the stabilization of these structures, however, the orientation of the (4aS, 7aS)-hexahydro-1H-pyrrolo[3,4-b]pyridin (hpb) ring determines the most stable structure. In Norfloxacin, the orientation of the ethyl group in the plane perpendicular to the ring frame determines the most stable structure whereas, the orientation of piperazine ring is critical for the determination of stable structure of the Norfloxacin zwitterion. Complexes of most stable structures of Moxifloxacin and Norfloxacin with one and two water molecules were also calculated. The Raman spectra were calculated for low energy structures of Moxifloxacin and Norfloxacin and also for their complexes with one and two water molecules. The experimental Raman spectra were obtained on powder sample of two molecules utilizing our lab-built Raman spectroscopy setup. The experimental Raman spectra were analyzed in light of the calculated Raman spectra of neutral molecules and their complexes with water. It was found that, in the powder form, the Raman spectra of two molecules can be better explained if intermolecular H-bonding with water molecules is considered. The study, therefore, confirms the hygroscopic nature of two molecules under ambient environment.

Keywords: Moxifloxacin, Norfloxacin, density functional theory, Raman spectroscopy.

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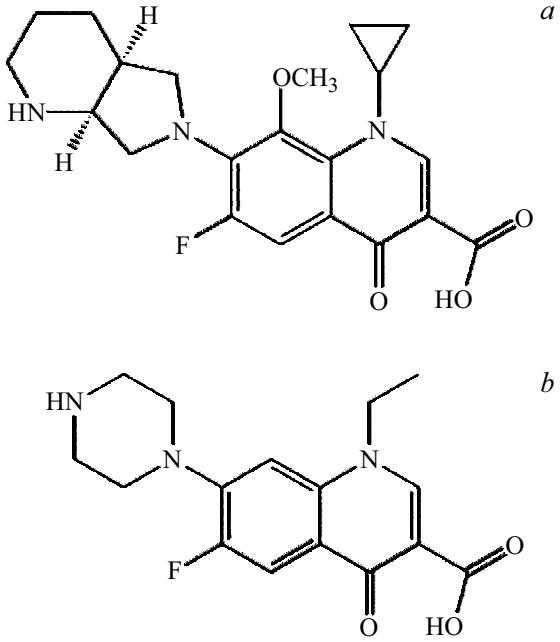
Introduction

The determination of the correct structure of molecules is of immense importance in chemistry as well as in biology to explain their structural and dynamical properties. In order to determine the biological activity of a biomolecule, correct structure determination becomes more demanding as their biological activity strongly depends on the structure and possible interaction sites [1]. Experimentally, vibrational spectroscopy techniques such as infra-red (IR) and Raman spectroscopy provide vital information on the vibrational modes in the molecules which are characteristic of the stable or unstable structure of the molecule. However, the spectroscopic information alone is not sufficient to obtain the correct structure of a molecule. Computational methods such as ab initio and density functional theory (DFT) facilitates the prediction of correct molecular structure by correlating the calculated vibrational frequencies with the spectroscopic signature obtained from the IR and Raman spectroscopy techniques [2].

Fluoroquinolone is a well-known class of molecule that consists of several drug molecules [3]. Moxifloxacin and Norfloxacin are two important Fluoroquinolone used in the treatment of several diseases. The chemical structures of two molecules are shown in Scheme 1.



Scheme 1. Chemical structure of (*a*) Moxifloxacin and (*b*) Norfloxacin.

Moxifloxacin has strong activity against gram-positive and anaerobic bacteria [4,5] whereas, Norfloxacin is known for

its antibacterial activities towards infectious diseases, such as gonorrhea and prostate and urinary tract infections [6,7]. These two molecules have been studied extensively for their biological activities, however, less attention was paid on the structure of two molecules or their complexes with water molecules. Neugebauer et al. presented the infrared and Raman spectra of Fluoroquinolone molecules, however, the work did not emphasize the structure of individual molecules [8]. The molecule Moxifloxacin has been detected in different mediums using surface enhanced Raman scattering (SERS) technique [5,9], however, the emphasis was not made on the structure of the molecule. The structure of Moxifloxacin has been studied using crystallographic methods and, several polymorphs of Moxifloxacin have been identified [10–17]. It was observed that the pharmaceutically important „form I" is anhydrous, however, it is hygroscopic and absorbs water during pharmaceutical processing to form more stable monohydrate form [10,18].

Norfloxacin has been studied for its interactions with metal ions and protein. Upadhyay et al. studied the Norfloxacin and its complexes with alkali metal perchlorates whereas Rusu et al. studied the Norfloxacin and its complex with silver ion using FT-IR spectroscopy [19,20]. Lian et al. have investigated the binding sites and orientation of Norfloxacin on bovine serum albumin (BSA) using SERS technique [21]. Huang et al. used the SERS technique to study the degradation of Norfloxacin from plasma produced ozone [22]. Among the study on structure, Vitorino et al. have performed the DFT calculations to study the structures of neutral and zwitterionic Norfloxacin complexes with water molecule [23] and, Sahoo et al. have studied Norfloxacin using Raman spectroscopy [24]. Although the structure of hydrated Norfloxacin has been studied, the possible structure is not correlated with the experimental vibrational spectra [23]. The structure of Norfloxacin has also been investigated through crystallography. Barbas et al. studied the polymorphs of Norfloxacin using FT-IR and Raman spectroscopy [25]. In the study, it was found that .form A. of the molecule is energetically more stable, however, it can pick moisture under ambient conditions compared to the less stable „form B" of Norfloxacin [25]. Although several studies were found in literature, the information on the correct molecular structure of Moxifloxacin and Norfloxacin and its correlation with IR or Raman spectra is missing.

In this work, the density functional theory calculated structure of Moxifloxacin and Norfloxacin is presented along with the H-bonded complexes of both the molecules with one and two water molecules. The structures of Moxifloxacin and Norfloxacin powder are discussed in light of calculated Raman spectra of two molecules, their complexes with one and two water molecules and, the experimental Raman spectrum obtained with our lab-built experimental setup. It appears through the study that the Raman spectra of Moxifloxacin and Norfloxacin powder are better understood when H-bonding interactions of Moxifloxacin and Norfloxacin with water molecule are considered.

Theoretical and experimental methods

The structures of Moxifloxacin and Norfloxacin were optimized using density functional theory. For the calculations, hybrid functional Becke 3-parameter Lee.Yang.Parr (B3LYP) was used along with the Gaussian type 6-31+G(d,p) basis set. Harmonic frequency calculation (with IR intensity and Raman activity) following the geometry optimization was also performed to ensure the molecule at the global minimum of the potential energy surface. The most stable structures of two molecules were subjected to the complex formation with water molecules. Structures of complexes with one and two water molecules were calculated along with their Harmonic frequencies. In the case of Norfloxacin, structures of anion and zwitterion were also calculated along with their Raman spectra. To compensate for the anharmonicity of the ground state potential, calculated Raman spectra were scaled with a factor of 0.97. All the density functional theory calculations were performed with the Gaussian09 program suite [26].

The Raman spectra of both molecules were recorded with the lab-built Raman spectroscopy setup. The Raman spectroscopy setup has been described in detail earlier [27,28]. Briefly, the setup uses an inexpensive 638 nm laser diode, a mirror with a hole in its center, a long pass filter, and a portable spectrometer. The laser diode wavelength is tunable. Both the power and wavelength of the laser diode were controlled by adjusting the operating current. This lab-built Raman spectroscopy setup provides the spectral resolution of $14-15\,\mathrm{cm}^{-1}$ in the $400-4000\,\mathrm{cm}^{-1}$ spectral region. The Raman spectra were recorded with 300 ms irradiation time and ten on-board averaging.

Results and discussions

Structures of molecules

Moxifloxacin and water complexes of Moxifloxacin. The structures of Moxifloxacin were calculated with B3LYP/6-31+G(d,p) theory level. Figure 1 shows the three low energy structures of Moxifloxacin. The atom numbers of non-hydrogen atoms are also shown in Fig. 1, *a*. The relative energies with respect to the most stable structure are given in the parentheses. All these structures show four intra-molecular H-bonds. It is known that the energy of molecular complexes have strong influence from H-bond lengths and energies [29,30]. The shortest (strongest) H-bond in all these structures is between O_1H and O_{28} atoms and the corresponding H-bond length is 1.67 Å. The other three H-bonds are formed by the interaction of O_{11} with C_6H and $C_{15}H$ and by the interaction of F_{24} with $C_{22}H$. These three H-bonds are of nearly similar strength. Despite the H-bonds of similar strengths in three structures, the energy of the structures MF-II and MF-III are 3.11 and 21.48 kJ/mol higher compared to the most stable structure MF-I. The observed major difference in these three structures is the change in the orientation of the

(4aS, 7aS)-hexahydro-1H-pyrrolo[3,4-b]pyridin (hpb) ring. This structural change could be a reason for the increase in energy of MF-II and MF-III structures compared to the energy of MF-I. The *xyz* coordinates of three structures are given in the Supplementary Tables S1−S3.

The complex of the water molecule with the most stable structure of Moxifloxacin (MF-I) is also investigated. Figures 2, *a*–*c* show the complex of MF-I with a single water molecule whereas Figs. 2, *d* and *e* show the complex of MF-I with two water molecules. It is evident from Figs. 2, *a*–*c* that water can bind with MF-I at either of three sites marked as A, B, and C in Fig. 1, *a*. When the water molecule is bound at site A, three intermolecular H-bonds stabilize the complex. The strongest H-bond is between O_{29} of MF-I with H-atom of the water molecule. The corresponding H-bond length is 1.8 Å. Other two H-bonds formed between O-atom of water with C_7H and C_4H with H-bond length of 2.288 and 2.354 Å, respectively. In isomer MF-I_W_2, a single H-bond is formed between O29 of MF-I and H-atom of water. This isomer is 12.86 kJ/mol higher in energy compared to the most stable isomer MF-I_W_1. When the water molecule acquires the binding site C, bidentate H-bond forms between the water molecule and O_{28} and $C_{25}H$ of MF-I. However, the energy of this isomer is 16.44 kJ/mol higher compared to the most stable structure.

In the complex of Moxifloxacin with two water molecules, the second water molecule always prefers to bind to site A and results in the most stable structure MF-I_2W_1 (Fig. 2, *d*). The energy of another 2-water complex, when one water molecule each occupies site A and site C, is 5.68 kJ/mol higher compared to the most stable structure MF-I_2W_1. In case when a water molecule is placed at site B, it always migrates towards site A resulting in the most stable isomer structure.

Norfloxacin and water complex of Norfloxacin. Figures 3, *a, b* show the two lowest energy structures of Norfloxacin optimized with B3LYP/6-31+G(d,p) theory level. The minimum energy structure has a planar quinolone ring frame with piperazine ring in the chair conformation. The ethyl group at N_5 atom is oriented in the plane perpendicular to the quinolone ring frame. In case when the ethyl group is in-plane with quinolone ring, the energy of the structure is raised by 5.21 kJ/mol resulting in the structure NF-II (Fig. 3, *b*). Both the structures NF-I and NF-II show intramolecular H-bonds of similar strength. Figures 3, *c* and *d* show the anion and zwitterion of structure NF-I. It was observed that the orientation of the piperazine ring changes dramatically in the zwitterion structure and form two similar strength H-bonds with F-atom. Four intermolecular interaction sites in the neutral Norfloxacin are marked as A, B, C, and D in structure NF-I shown in Fig. 3, *a*. The *xyz* coordinates of these structures are given in the Supplementary Tables S4−S7.

Investigation of structure of the water complexes with the most stable structure NF-I was also carried out. Figures 4, *a*–*d* show the one water complex of NF-I when the water molecule is bound to A, B, C, or D binding sites. The length of the resulting H-bond is also shown as dotted lines. The complex is most stable when the water molecule is bound to site A. The energies of other single water complexes are 10.14, 12.86, and 14.80 kJ/mol higher when the water molecule is accommodated at sites B, C, and D, respectively. In the case of the complex of the most stable structure of Norfloxacin with two water molecules, three low energy structures are shown in Figs. 4, *e*–*g*. The minimum energy structure accommodates two water molecules at binding site A in a ring fashion. The next structure (NF-I_2W_2) where one water each are accommodated at binding site A and B is 12.4 kJ/mol higher in energy. The third structure accommodates one water molecule each at binding sites A and D and is 17.3 kJ/mol higher in energy compared to the most stable structure.

Raman spectroscopy of Moxifloxacin and Norfloxacin powder

Moxifloxacin The Raman spectrum of Moxifloxacin was obtained using our lab-built Raman spectroscopy set up. Figure 5, *a* shows the Raman spectrum of Moxifloxacin powder. In this experimental Raman spectrum, clear bands were observed at 1706, 1612, 1549, 1420, 1371, 1349, 823, and 543 cm^{-1}. Apart from these prominent bands, shoulder bands at 1310 and $\sim$1284 cm^{-1} are also observed. The peak positions of these bands are listed in Table 1.

Figure 5, *b* shows the calculated Raman spectrum of the most stable structure of Moxifloxacin MF-I. The peak positions of the scaled frequencies are listed in Table 1 along with the mode assignments. As it is evident, all the bands observed in the experimental Raman spectrum (Fig. 5, *a*) are in good agreement with the calculated peak position except for the bands observed at 1706 and 1549 cm^{-1} in the experimental Raman spectrum. The band at 1706 cm^{-1} corresponds to $C_2{=}O_{29}$ stretching mode whereas the band at 1549 cm^{-1} is possibly due to the $C_{27}{=}O_{28}$ stretching vibration. The strong shift of 1549 cm^{-1} could be a result of intramolecular H-bond (H-bond length: 1.670 Å) between O_1H and O_{28} atoms. The band at 1612 cm^{-1} is largely due to the C−C stretch of the pyridine ring frame. The band at 1420 cm^{-1} is due to the symmetric bending of C−H of methyl group and the CH_2 of cyclopropane ring. The less resolved spectral region 1250−1380 cm^{-1} shows clear band maxima at 1371 and 1349 cm^{-1}. These bands correspond to the C−H in-plane bend and CH2 wagging modes respectively. Besides these two clearly visible bands, shoulder bands were also observed at 1310 and $\sim$1284 cm^{-1}. These two bands match well with the C−N and C−C stretches as listed in Table 1. Other two prominent bands in the Raman spectrum are observed at 823 and 543 cm^{-1}. These bands correspond to CH_2 wagging and CCC bending modes.

The spectral shift observed in bands at 1706 and 1549 cm^{-1} could be a result of intermolecular H-bonding.

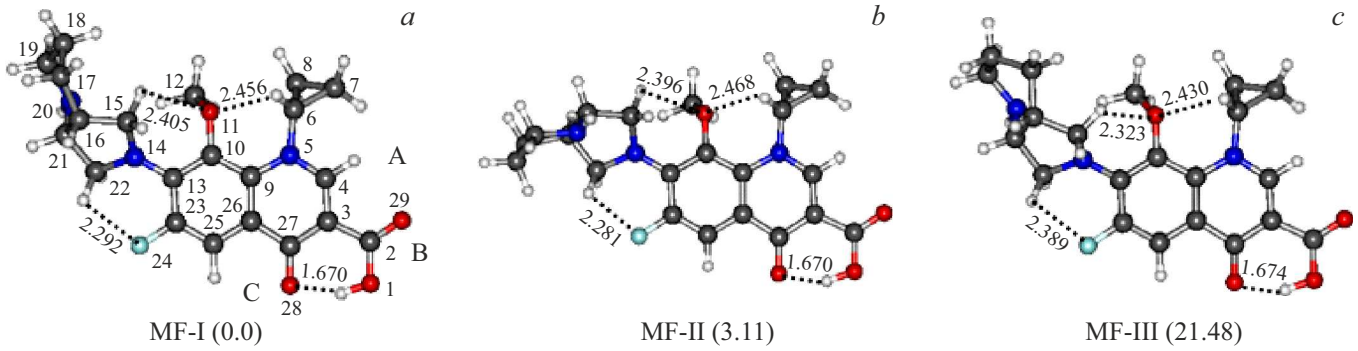


Figure 1. B3LYP/6-31+G(d,P) calculated structures of Moxifloxacin. The energies (in kJ/mol) relative to the most stable structure are given in parentheses. H-bond lengths are in Å. Intermolecular binding sites (A, B and C) and atom numbers are shown in (*a*).

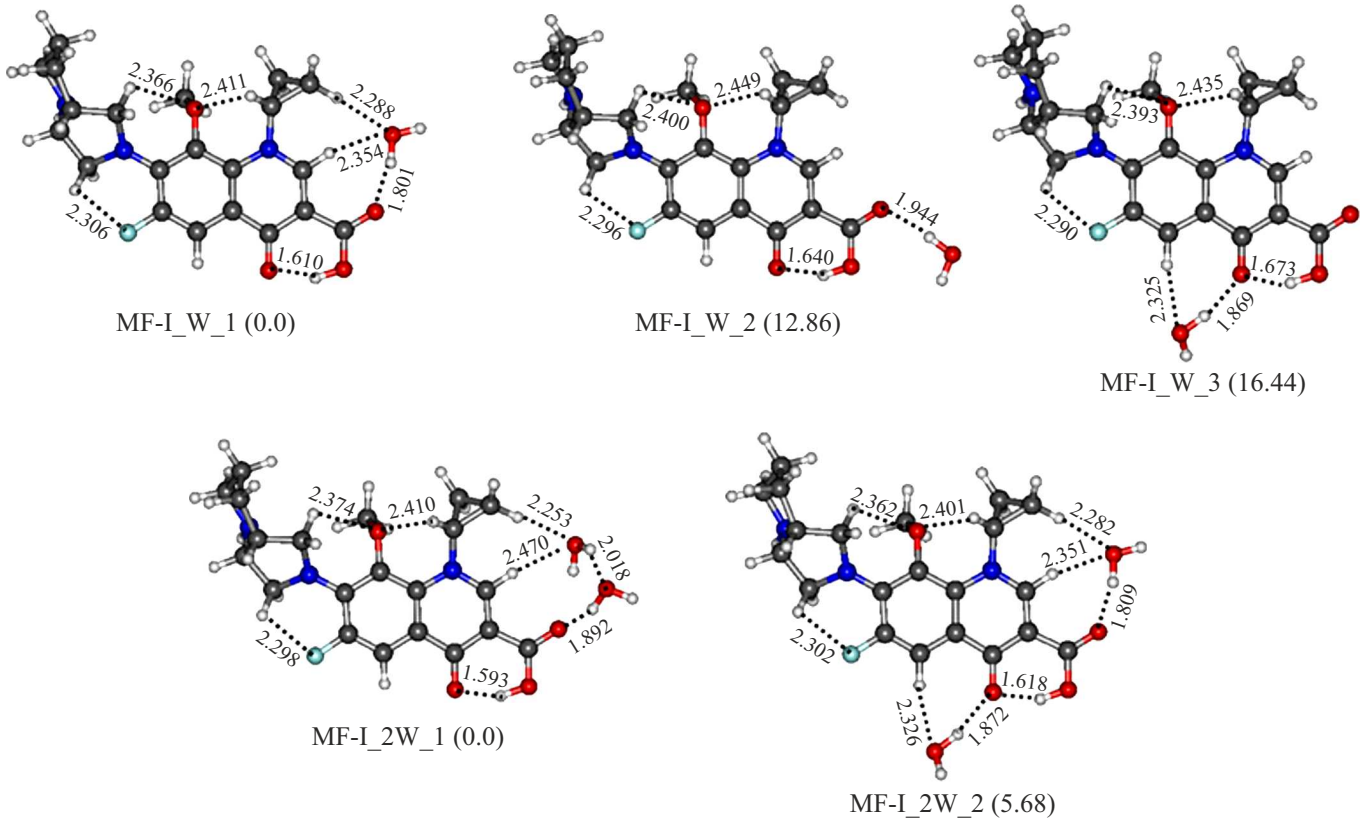


Figure 2. Optimized structures of Moxifloxacin-water complexes: (*a*)–(*c*) with one water molecule and (*d*)–(*e*) with two water molecules. The energies (in kJ/mol) relative to the most stable structure are given in parentheses. H-bond lengths are in Å. The calculations were performed at B3LYP/6-31+G(d,p) level.

In order to investigate the effect of H-bonding, the Raman spectrum of MF-I with one and two water molecules was also investigated. The corresponding calculated Raman spectra are shown in Figs. 5, *c* and *d* for structures MF-I_W_1, and MF-I_2W_1 shown in Fig. 2. The scaled normal mode positions are also listed in Table 1. As it can be seen, the H-bonding of MF-I with one water molecule at binding site A (MF-I_W_1) shift the $C_2{=}O_{29}$ stretching mode from 1744.5 cm^{-1} in neutral molecule to 1718.5 cm^{-1} and, another water molecules at the same binding site shifts the calculated band position further to 1709.9 cm^{-1}, which is in excellent agreement with the experimentally observed band. For the experimentally observed band at 1549 cm^{-1}, the calculated band positions are 1565.2, 1557.9, and 1554.8 cm^{-1} for MF-I, MF-I_W_1 and MF-I_2W_1, respectively. In this case also, it is clear that the complex formation at binding site A modulates the position of $C_{27}{=}O_{28}$ stretching mode leading it to be in better agreement with the experimentally observed band. With these observations on the effect of H-bonding on C=O

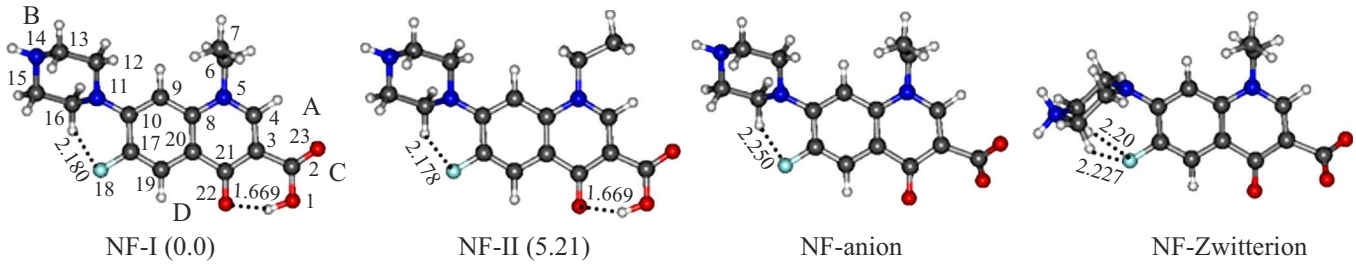


Figure 3. Optimized structures of Norfloxacin: (*a*), (*b*) neutral, (*c*) anion and (*d*) zwitterion. The energies (in kJ/mol) relative to the most stable structure are given in parentheses. H-bond lengths are in Å. The calculation was performed at B3LYP/6-31+G(d,p) level.

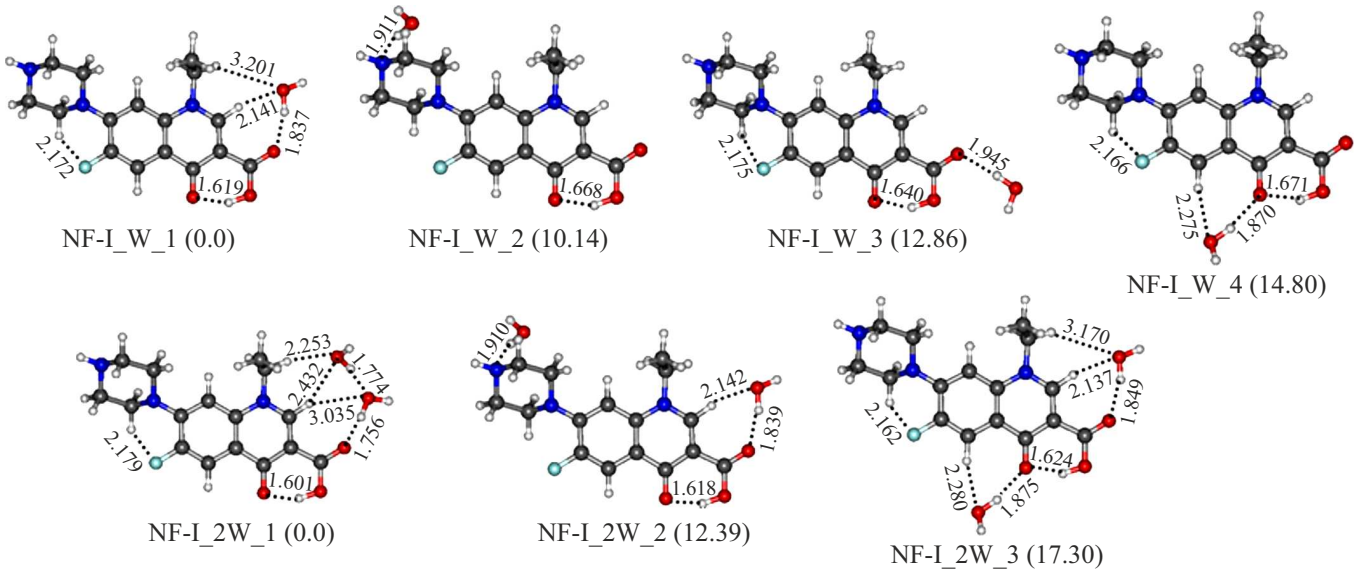


Figure 4. Optimized structures of Norfloxacin-water complexes (*a*)–(*d*) and Norfloxacin-2water complexes (*e*)–(*g*). The energies (in kJ/mol) relative to the most stable structure are given in parentheses. H-bond lengths are in Å.

stretching mode, it is evident that the Raman spectrum of Moxifloxacin can be better understood by considering the intermolecular H-bonding. In the supplementary Fig. S1 calculated Raman spectra of structure MF-I, MF-II, MF-III, MF-I_W_1, MF-I_W_2, MF-I_W_3, MF-I_2W_1, and MF-I_2W_2 are shown along with the experimental Raman spectrum. It is evident from the figure that the closest agreement for two C=O modes has appeared for the structure MF-I_W_1.

Although study on the molecular structure of Moxifloxacin in isolation is not well reported in the literature, crystal structure studies reveal several polymorphs of Moxifloxacin. It has also been shown that Moxifloxacin appears in both anhydrous and hydrated forms. The anhydrous form is found to be hygroscopic and absorbs water molecules [18]. Grunenberg et al. have shown the Raman spectra of the anhydrous and monohydrated form [10] where a clear shift in the position of C=O stretching mode can be seen in monohydrated form upon careful inspection. The Raman spectrum presented in this work and its interpretation in light of calculated spectra also shows that the Raman spectrum is better explained when a complex of Moxifloxacin with water molecule is considered. This confirms the hygroscopic nature of the Moxifloxacin in ambient environmental conditions.

Norfloxacin The experimentally obtained Raman spectrum of Norfloxacin in the fingerprint spectral region is shown in Fig. 6, *a*. In contrast to the Raman spectrum of Moxifloxacin, vibrational features in the spectrum are clearly resolved. In the spectrum, clear bands were observed at 1720, 1621, 1584, 1466, 1390, 1324, 1263, and 854 cm^{-1}. These bands are listed in Table 2 presented below. In order to identify the normal modes and resemblance with the experimental Raman spectrum, in Figs. 6, *b*–*d*, calculated Raman spectra of the most stable Norfloxacin isomer (NF-I), and the most stable isomer of its complex with one and two water molecules are also presented. The band positions observed in these calculated spectra along with their assignment are given in Table 2. It is clearly evident from the figure and table that the calculated band positions of NF-I are in very good agreement with the bands observed in the experimental Raman spectrum except

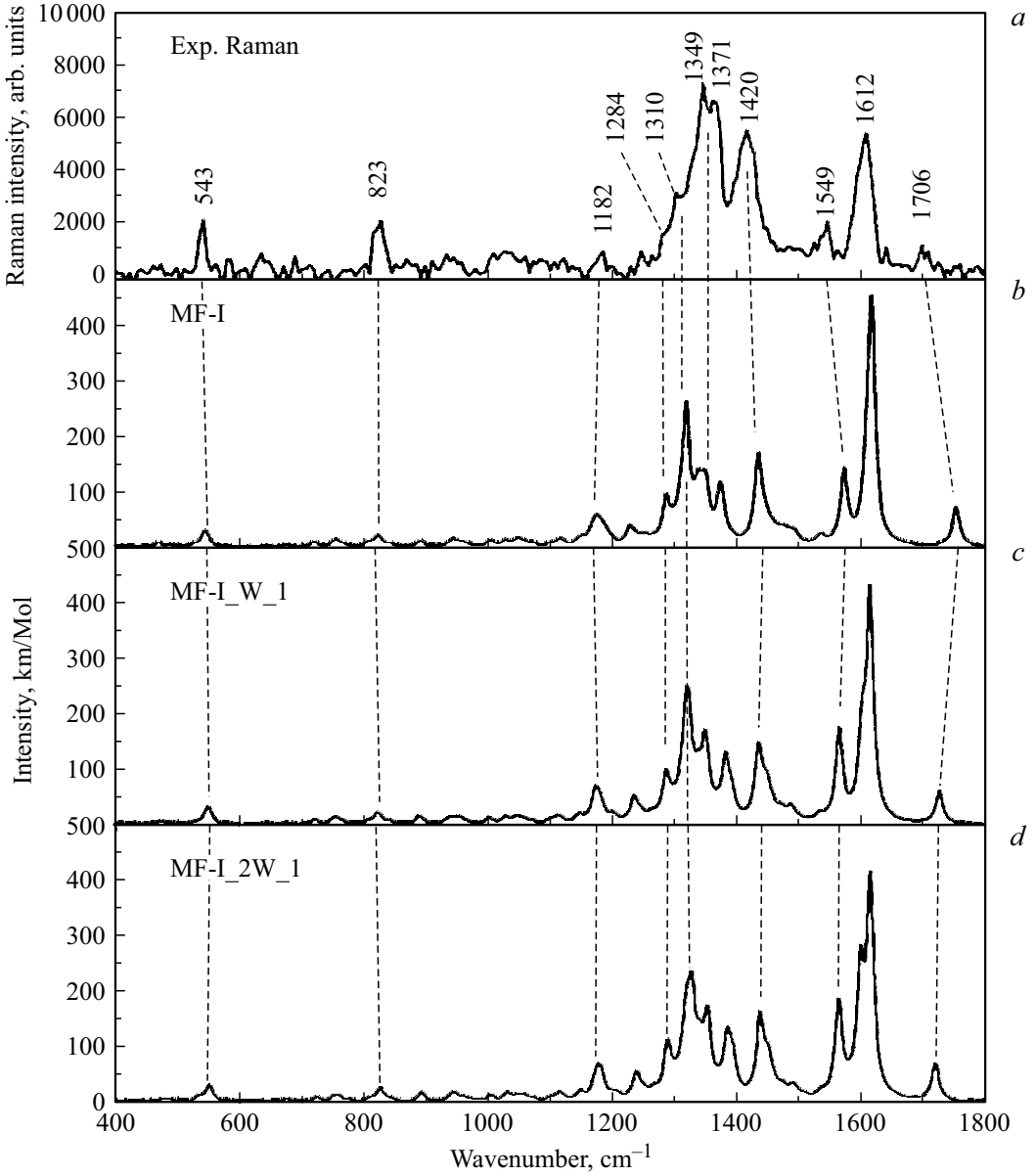


Figure 5. (*a*) Experimental Raman spectrum and calculated Raman spectrum of Moxifloxacin for (*b*) structure MF-I, (*c*) structure MF-I_W_1, (*d*) structure MF-I_2W_1. The calculated spectra are scaled with 0.97.

for the band at 1720 and 1324 cm^{-1}. The band observed at 1720 cm^{-1} corresponds to stretching of $C_2{=}O_{23}$ and in-plane bending of O_1–H whereas the band at 1324 cm^{-1} is a combination of several modes such as in-plane bending of C_4H, stretching of C_2–C_3, stretching of N_5–C_6, and ring breathing. In the calculated spectrum of NF-I, these bands appeared at 1756 and 1299 cm^{-1} respectively. Since C_2O_{23} is the most preferred site for intermolecular binding, the stretching frequency may get altered in the case of H-bonding to a water molecule. As it can also be seen in Figs. 6, *c*–*d* and Table 2, the C=O stretching mode shifts to 1733 upon complex formation with one water molecule and to 1725 cm^{-1} upon complexation with two water molecules. The stretching frequency obtained with two water molecules is in excellent agreement with the experimental band at 1720 cm^{-1}. It has to be noted that in NF-I_2W_1, two water molecules are H-bound to site A. In case when one water molecule is bound to site A and another at site B, this stretching frequency is 1722 cm^{-1}. This clearly shows that the presence of water molecule at site A has a profound effect on the frequency of C=O stretching mode and the band at 1720 cm^{-1} can be understood better if H-bonded water is considered. Upon complexation with water molecules, the mixed mode appeared in NF-I at 1299 cm^{-1} also shifts to blue, and appeared at 1305 cm^{-1}, which is in better agreement with the experimental band observed at 1324 cm^{-1} in the experimental Raman spectrum. Raman spectrum of Norfloxacin along with calculated spectra for

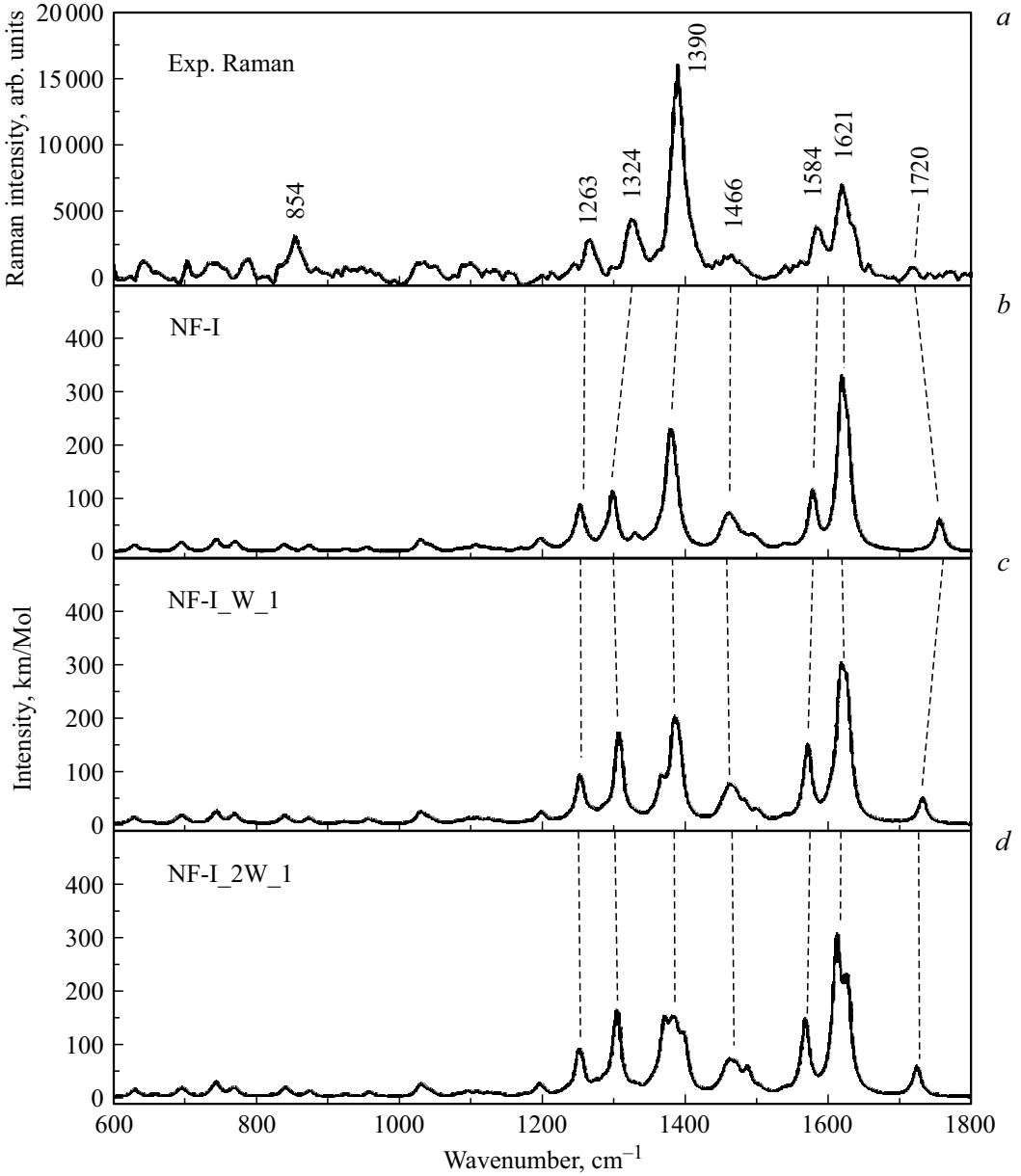


Figure 6. (*a*) Experimental Raman spectrum and calculated Raman spectrum of Norfloxacin for (*b*) structure NF-I, (*c*) structure NF-I_W_1, (*d*) structure NF-I_2W_1. The calculated spectra are scaled with 0.97.

neutral, anionic, and zwitterion structures are shown in Supplementary Fig. S2. Supplementary Fig. S3 shows the experimental Raman spectrum of Norfloxacin with calculated Raman spectra for one and two water complexes of Norfloxacin.

Compared to Moxifloxacin, Norfloxacin has been studied in more detail in the literature. In the Raman spectrum, Neugebauer et al. observed a C=O band at 1722 cm^{-1} for Norfloxacin in the solid-state. The authors observed that upon hydration this characteristic band disappeared possibly due to the formation of anionic or zwitterionic structure. However, it is important to note that the hydration of molecules was performed by mixing anhydrous molecule with water in a ratio of 1 : 6. Therefore, a significantly higher number of water molecules may have interacted with the fluoroquinolone molecule leading to the formation of anionic or zwitterionic structure. In recent work by Lian et al., the C=O band was observed at 1722 cm^{-1}, however, it was not predicted by their calculation, perhaps due to the incorrect molecular structure [21]. In the normal Raman spectrum of Norfloxacin Huang et al. also observed a band at the same position [22]. Although these authors have presented the Raman spectrum of Norfloxacin, the correlation of spectra with the molecular structure was not emphasized. In the crystal structure studies, it was found that the molecule Norfloxacin is hygroscopic, and possibly undergoes hydration in the ambient environment [10,25]. In the study above, we correlated the Raman spectrum of

Table 1. Experimentally observed Raman bands with calculated position of normal mode (cm^{-1}) and their assignments for Moxifloxacin, the calculated band positions were scaled with a factor of 0.97

Exp.	MF-I	MF-I_W-1	MF-1_2W_1	Assignments
1706	1744.5	1718.5	1709.9	$C_2{=}O_{29}$ stretch, O_1–H in-plane bend
1612	1608.8	1607.3	1605.6	C−C stretch benzene ring
	1600.5	1594.6	1590.0	C−C stretch benzene ring + $C_{27}{=}O_{28}$ stretch
1549	1565.2	1557.9	1554.8	$C_{27}{=}O_{28}$ stretch, C_4H in-plane bend, O_1H in-plane bend
1420	1426.4	1428.4	1428.8	symmetric C−H bend of CH_3 (umbrella mode) + CH_2 scissoring in cyclopropane ring
1371	1366.9	1376.2	1377.6	C_4H and C_6H in-plane bend
1349	1343.5	1345.0	1345.3	CH_2 wagging of $C_{15}H_2$ and $C_{22}H_2$
	1331.0	1331.6	1332.2	CH_2 wagging of $C_{15}H_2$ and $C_{22}H_2$
1310(sh)	1312.1	1317.7	1319.5	C_4N_5 stretch + C_2C_3 stretch + $C_{26}C_{27}$ stretch
		1312.0	1310.6	
1284(sh)	1280.1	1280.9	1281.0	$C_{10}C_{13}$ stretch + $C_{25}C_{26}$ stretch + $C_{22}H_2$ wagging
1182	1182.9	1176.7	1177.0	Asymmetric CH bend
	1176.7	1172.7	1172.7	Asymmetric CH bend
	1172.3	1167.9	1170.9	Asymmetric CH bend
	1167.4	1167.0	1168.6	Asymmetric CH bend
	1160.1	1164.0	1163.9	Asymmetric CH bend
823	818.1	818.2	819.1	C_8H_2 and C_7H_2 wagging
543	542.1	546.4	546.6	$C_{10}C_{13}C_{23}$ and $C_{17}C_{18}C_{19}$ bend

Table 2. Experimentally observed Raman bands with calculated position of normal mode (cm^{-1}) and their assignments for Norfloxacin, the calculated band positions were scaled with a factor of 0.97

Exp.	NF-I	NF-I_W-1	NF-I_2W_1	Assignments
1720	1756.1	1733.1	1724.8	$C_2{=}O_{23}$ stretch, O_1–H in-plane bend
1621	1627.4	1627.3	1626.9	C−C stretch benzene ring
	1618.5	1618.1	1612.6	C−C stretch pyridone ring
1584	1578.3	1572.3	1568.4	$C_{21}{=}O_{22}$ stretch, C_4H in-plane bend, O_1H in-plane bend
1466	1462.4	1463.4	1463.3	CH_2 symmetric bend (scissoring mode) in ring
	1452.4	1453.6	1453.9	CH_2 symmetric bend (scissoring mode) in ring + O_1H in-plane bend
1390	1383.0	1393.2	1398.7	CH_2 wagging + CH symmetric bend of CH_3 group
	1377.5	1388.3	1388.1	CH_2 wagging + C_4H in-plane bend + C−C stretch of $C_8{-}C_{20}$
		1383.7	1381.0	CH_2 wagging
		1367.3	1370.7	CH_2 wagging + C−C stretch
1324	1298.6	1307.7	1304.6	C_4H in-plane bend + C_2–C_3 stretch + N_5–C_6 stretch + ring breathing
1263	1252.4	1254.4	1252.7	CH in-plane bend + CH_2 twisting

Norfloxacin with various calculated structures. As it was observed, the spectral features are in better agreement with the calculated spectra when the inclusion of one or two water molecules at site A is considered. This observation confirms the hygroscopic nature of Norfloxacin.

Conclusions

We have studied the structure of Moxifloxacin and Norfloxacin powders using our home-built Raman spectroscopy setup and the DFT calculations. It was found that the energies of structures of Moxifloxacin are largely governed by the orientation of the hpb ring. In the case of Norfloxacin, the stable structure is formed when the ethyl group is oriented perpendicular to the plane of the ring frame. Both the molecules show multiple intermolecular interaction sites for the H-bonded interaction with water molecules. The complexes of molecules with one and two water molecules were also investigated using the DFT. The most stable structure in both the molecules, forms when the water molecule interacts through the binding site „A“. This structure also has a profound effect on the frequencies of the C=O stretching modes leading to better interpretation of the experimentally obtained Raman spectrum. The outcome of the present study will be extremely useful in the study of molecular complexes of two molecules.

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Conflict of interest

The author declares that there are no conflict of interest.

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