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Spectroscopic signatures and structural motifs in isolated and hydrated caffeine: a computational study

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Abstract:

The conformational landscapes of neutral caffeine and its hydrated complex have been investigated by MP2 and DFT methods. The ground state geometry optimization yield six lowest energy structures for bare caffeine and five lowest energy conformers of caff$_1$-(H_2O)$_1$ complex at the MP2/6-311++G(d,p) level of theory for the first time. We investigated the low-lying excited states of bare caffeine by means of coupled cluster singles and approximate doubles (CC2) and TDDFT methods and a satisfactory interpretation of the electronic absorption spectra (Phys. Chem. Chem. Phys., 2012,14,10677-10682) is obtained. The difference between the S_0-S_1 transition energy due to the most stable and the least stable conformation of caffeine was found to be ~ 859 cm^{-1}. One striking feature is the coexistence of the blue and red shift of the vertical excitation energy of the optically bright state S_1 ($^1\pi\pi^*$) of caffeine upon forming complex with a water at isolated and conjugated carbonyl sites, respectively. The lowest singlet $\pi\pi^*$ excited-state of the caff$_1$-(H_2O)$_1$ complex involving isolated carbonyl are strongly blue shifted which is in agreement with the result of R2PI spectra of singly hydrated caffeine (J.Chem. Phys. 2008, 128, 134310). While for the most stable and the second most stable caff$_1$-(H_2O)$_1$ complexes involving conjugated carbonyl, the lowest singlet $\pi\pi^*$ excited-state is red shifted. The effect of hydration on S_1 ($^1\pi\pi^*$) excited state due to bulk water environment was mimicked by a combination of polarizable continuum solvent model (PCM) and conductor like screening model (COSMO), which also shows a blue shift in accordance with the result of electronic absorption spectra in aqueous solution (Phys. Chem. Chem. Phys., 2012,14,10677-10682). This hypsochromic shift, is expected to be the result of the changes in the π-electron delocalization extent of molecule because of hydrogen bond formation.

1. Introduction

The understanding of the photo physical and photochemical properties of nucleic acid bases, the most fundamental building blocks of life, is essential for the rationalization of the photo stability as well as photodamaze mechanism of DNA and RNA.[1] As UV radiation can cause serious damage to DNA, leading to modifications in the genetic code, this topic is highly important. In the last decade, photochemistry of nucleic acid bases and related molecular systems have been extensively studied with experimental and theoretical methods.[1-12] A striking feature of the DNA and RNA bases is the efficient deactivation through very fast radiationless decay processes back to the electronic ground state after absorption of UV light which is believed to prevent the bases from destructive photochemical reactions.[1,6] In this regard photo dynamics of nucleic acid analogue, xanthine (3,7-dihydro-purine-2,6-dione), plays an important role with the goal of transferring the experience obtained in these simpler systems to more complex situation found in DNA. In the gas phase, xanthine and its methyl derivatives can exist in a variety of different tautomeric forms, which can exhibit drastically different photo physical behavior. Resonant two photon ionization (R2PI) experiment performed for the methylated derivatives of xanthine (caffeine, theophylline and theobromine) in the gas phase[8] were all sharp and vibronically resolved, suggesting a long excited state lifetime. Recently, Chen and Kohler[9] experimentally performed the femtosecond transient absorption spectroscopy of the same compounds in water and acetonitrile with the same excitation wavelength (266 nm) as used in R2PI spectra and the results suggest, however , that these species relax to the electronic ground state on a subpicosecond time scale. Caffeine (1,3,7-trimethyl-Xanthine), the most widely consumed psychoactive stimulant[12-14] in the world, has the shortest life time (0.54 ps)[9] in the studied lowest excited state ($^1\pi\pi^*$) among all the methyl xanthine compounds.[9] Therefore, caffeine as well as other methyl xanthines could have been photostable building blocks in prebiotic environments exposed to intense UV radiation.[9] Nachtigallova et al[10] studied the excited state behavior of methylated xanthines and their computed relaxation paths from the lowest excited state S_1 minima towards conical intersections indicates that at least part of the population will be retained in the excited state so that it will be detected in the R2PI experiment as sharp vibrationally resolved spectra, which is in agreement to earlier report.[8]

Spectroscopic signatures of isolated biomolecules and their hydrated clusters may provide insight on their preferred conformations, dynamical flexibility, and inter- and intra- molecular interactions determining their skeletal structures. Therefore, it is a great challenge to measure the spectral signatures and consequently to extract the contributions of the conformational isomers, while assigning them to specific structures. A systemic search of the conformers of caffeine molecule requires consideration of rotations of methyl groups. The excited states of caffeine are important in understanding photophysics and photochemistry of caffeine-DNA complexes. In the isolated caffeine, the lowest excited state S_1 exhibits $\pi\pi^*$ character and primary photo-excitation involves this optically bright state S_1 ($^1\pi\pi^*$). It is common knowledge that hydration can shift electronic states and hence, it can modify the excited state dynamics. Microsolvation can open new relaxation channels[15] and hydrogen bond formation can affect the π-electron structure of particular isomers of a biomolecular complex.[16] Kim et al[11] studied the excited states of caffeine and its hydrated complexes in a supersonic jet by resonant two photon ionization (R2PI) and UV-UV hole burning spectroscopy, and concluded that the short life time of the excited state is responsible for the anomalous distribution of caffeine water clusters. They have also carried out conformational analysis for bare and hydrated caffeine by density functional theory (B3LYP)[17-19] calculations, however result of their calculations seems to be ambiguous and incomplete.[11] Unfortunately, while the few experimental results have been obtained for caffeine, none of the few computational papers including solvent effects takes into account employing the Moller-Plesset perturbation theory method.[20] In quantum mechanical computations, a high degree of electron correlation must be included to reliably account for dispersion interaction. Second order Moller-Plesset perturbation theory method (MP2)[20] offers a better approach for describing non-covalent interactions, since it can be extended to much larger systems. For assignment of the observed R2PI electronic spectra of caffeine and its monohydrated clusters, a systematic quantum mechanical computation employing more reliable approach is also required. Second order Moller-Plesset perturbation theory (MP2),[20] second order approximate coupled cluster (CC2)[21]and density functional theory (DFT)[17-19,22] methods implemented in Gaussian[23] & TURBOMOLE[24] quantum chemical software's, provide important insights into the energetics, ground state structures and photochemistry of these systems. Our aim is to investigate most of the stable structural motifs of caffeine and its hydrated complexes. Furthermore, we will investigate the low-lying excited states as well as the effect of hydration on the lowest singlet $\pi\pi^*$ excited- state of caffeine. The observed blue shifted bands in R2PI spectra of singly hydrated caffeine complex are explained and the possible reasons of missing of the red shifting bands will be discussed in view of the earlier experimental and theoretical reports. The application of DFT to non-covalently bound complexes has been limited due to the failure of most density functional approximation, in many case, to describe dispersion interaction. However, several approaches exist for improving existing density functionals to handle dispersion effects. In this paper, we also report a comparative study of the accuracy of the B3LYP[17-19], B3PW91[17-19, 25] and X3LYP[18,26] density functionals and newly developed M06, M06-2X[27,28] and DFT-D functionals [29] to predict the energy and/or binding energy of caffeine and its monohydrated complexes.

2. Computational Methods

Electronic structure calculations have been performed using the Gaussian 09[23] & TURBOMOLE V6.4 [24] quantum mechanical software packages. For bare caffeine, all possible rotamers (conformers) resulting from the rotation of methyl groups were initially optimized by the Hartree-Fock method employing the lower basis set. Finally all six ground state molecular structures of caffeine have been optimized using the second order Moller-Plesset perturbation theory (MP2)[16] and density functional theory[15] employing the B3LYP,[17,23-24] B3PW91,[17,23-25] X3LYP[23,26] and newly developed M06 and M06-2X[27,28] exchange-correlation functionals. The ground state structure of the most stable conformer of caffeine (Figure 1a) was also optimized using resolution of identity (RI) second order approximate coupled cluster (CC2)[19] and DFT-D[29] implementation in TURBOMOLE V6.4 employing the basis set TZVP. For monohydrated caffeine, all possible locations of interactions between caffeine and water were considered, such as oxygen atom in the carbonyl groups (isolated and conjugated carbonyls), the nitrogen atom, and pi cloud of the ring. The lowest energy conformers were selected from these initial geometries by using the HF method, while their geometry finally optimized by using most of the above methods employed for bare caffeine. Theoretical methods described above were applied to a molecular geometry without symmetry restrictions. The calculated geometry with C_1 symmetry was very close to that with C_s symmetry. All optimized structures have been verified as minima by performing frequency calculations in order to ensure that no imaginary frequency were present.

The CC2 method is an approximation to the coupled cluster singles and doubles (ccsd) method where single equations are retained in the original form and the double equations are truncated to the first order in the fluctuating potential. X3LYP [26](extended hybrid functional with Lee-Yang-Parr correlation functional[21]) extended functional for density functional theory was developed to significantly improve the accuracy for hydrogen bonded and van der waals complexes. The M06 and M06-2X [27,28] are newly developed standard hybrid DFT functionals with parameters optimized on training sets of benchmark interaction energies. According to Zhao and Truhlar,[27] the M06 series of functionals represent a significant step forward in density functionals, implicitly account for 'medium- range' electron correlation, which is sufficient to describe the dispersion interaction within many complexes. The 'medium- range' correlation is defined to be that found in complexes separated by about 5 A^0 or less so these functionals are fully benchmarked for biologically relevant non covalent interactions.[28]

DFT calculations employing the B3LYP and B3PW91 functionals [17-19,25] were found to produce the optimization yielded structures with one low imaginary harmonic wave number. However MP2 optimized geometries were found to produce only real harmonic wave numbers in all cases. The fundamental frequencies calculated by DFT method with B3LYP parameterization using the MP2 optimized geometries are significantly more accurate than those produced by using the DFT optimized geometries. The 6-31+G(d), and 6-311++G(d,p) basis sets in Gaussian 09 were mainly employed in the geometry optimization and vibrational modes calculation of caffeine and its hydrated clusters. TD-DFT method[22] employing B3LYP[17-19] and CAM-B3LYP[30] functional with 6-311++G(d,p) basis set was used at corresponding (DFT-B3LYP) ground state optimized geometries to predict the electronic absorption wavelengths. CAM-B3LYP[30,31] is a hybrid exchange-correlation functional which combines the hybrid qualities of B3LYP and the long range correction presented by Tawada et al[32], performing well for charge transfer excitations. RI-CC2[19,22] implementation in TURBOMOLE V6.4,[22]employing the basis set TZVP is also used to compute the vertical excitation energies (VEE) of the lowest energy conformer of bare caffeine , using CC2/MP2 optimized geometry. The effect due to the so-called bulk water molecules was taken into account within the polarizable continuum model (PCM)[33-35] and the conductor like screening model (COSMO)[36] framework. The electronic absorption spectra in aqueous solution have been calculated by employing the B3LYP hybrid exchange correlation functionals, using the PCM and COSMO continuum solvent models.[33-38] Binding energies (ΔE_{Stab}) of the caffeine -water complexes have been calculated as follows:

$$\Delta E_{\text{Stab}} = E_{\text{Complex}} - (E_{\text{caffeine}} + E_{\text{Water}})$$

The calculated binding energy of caffeine-(water)$_1$ complexes is corrected for the basis set superposition error (BSSE), using the counterpoise method of Boys and Bernardi.[39]

3. Result and discussion

3.1. Ground-state optimized geometries of caffeine

The conformational flexibility of caffeine is mainly arising from rotations of methyl groups. While there have been a previous computational study[11] on the conformational preferences of caffeine, in which four conformers of caffeine were determined and all were found within 0.5 kcal/mol (2.09 KJ/mole).[11] Which means that all of four conformers may relax to a single conformer because of very low energy barrier.[11] This calculation was carried out using only DFT-B3LYP and seems to be incomplete and ambiguous. We therefore sought to carry out a complete conformational search using MP2 method and other DFT procedures outlined in the earlier section. The geometry optimization of possible conformers in the ground state of caffeine using MP2 and DFT (M06, M06-2X, X3LYP, B3LYP, and B3PW91) methods employing the basis set 6-311++G(d,p), yield six most stable structures. All the six optimized structures of bare caffeine resulting from the rotation of methyl groups at MP2/6-311++G(d,p) level are shown in Fig.1. The relative energies of these six conformers at various levels of theory were summarized in Table 1. The total energy and dipole moment of these conformers at various levels of theory and the corresponding structural parameters at MP2/6-311++G(d,p) level are given in Table S1 and Table S2, respectively , of the ESI. As seen in Table 1, the relative energy obtained by DFT employing M06-2X functional is found more near to MP2 value in comparison to other DFT (X3LYP, B3LYP, B3PW91) values. At each level of theory calculated most stable rotamer was assigned as conformer A, the second most stable as conformer B, the third, fourth, fifth and sixth most stable were assigned as conformer C, D, E and F respectably as seen in Fig 1.The relative energy between the most stable conformer A and the least stable conformer F of caffeine was found to be 5.99, 4.95, 4.29 and 3.65 KJ/Mole at MP2/6-311++G(d,p), M06-2X/6-311++G(d,p), M06/6-311++G(d,p) and B3LYP/6-311++G(d,p) levels (See Table 1) respectively, which is relatively more larger than the value 1.84 KJ/Mol reported by Kim et al.[11] (at B3LYP/6-311++G(d,p level). Even the B3LYP value of Kim et al.[11] is found different from our calculation. Thus the earlier prediction that caffeine has only four rotamers[11] and they are all within 0.5 kcal/mol (2.09 KJ/mole)[11] seems to be incorrect. As seen in Table 1, the MP2 values strongly support the independent existence of various conformers of caffeine, therefore the possibility of more than one stable conformer of caffeine in the gas phase cannot be excluded. The total energy and dipole moment (in bracket) of the ground state optimized structure of the most stable conformer A of bare caffeine at RI-CC2/TZVP, RI-MP2/TZVP and DFT-D/TZVP levels using TURBOMOLE V6.4 were found to be -679.0213706 A.U.(3.78 D), -678.9768054 A.U.(3.82 D) and -680.2475456 A.U.(3.94 D) respectively, which are approximately in consonance to values given in Table 1. The optimized geometry of the most stable conformer A of caffeine at each level of theory is found similar to the geometry shown by Farrokhpour and Fathi.[40]

Caffeine has two carbonyl groups, C_2-O_{11} (isolated carbonyl) and C_6-O_{13} (conjugated carbonyl), joined to N_1 atom, as shown in Fig. 1 . The bond length of conjugated carbonyl of each conformer, 1.228-1.229 A^0, is found slightly greater than that of isolated carbonyl, 1.223-1.224A^0, at MP2/6-311++G(d,p) level (See Table S2 of the ESI). Similar trend was also found with DFT calculations. Difference in the bond lengths of the optimized geometry in all six conformers of bare caffeine were found to be less than 0.01 A^0, however significant changes were found at bond angles C2-N3-C12 and C4-N3-C12. The total electron density of caffeine indicates a build-up of charge density on the oxygen and nitrogen atoms and nodes at the other atoms. The NBO calculations at the MP2/6-311++G(d,p) level of theory led to negative charge densities of -0.731, -0.726, -0.624 and -0.610 in caffeine A on O_{11}, O_{13}, N_1 and N_9 atoms respectively (See Table S3 of the ESI). One can note that the negative charge densities on the N_3 and N_7 atoms are also large , but not as significant as on the oxygen atoms. The other caffeine conformers led to similar values.

We have computed the harmonic vibrational frequencies and intensities of the six caffeine conformers. Calculated DFT-B3LYP/6-311++G(d,p) vibrational wave numbers are found to be slightly larger than the fundamental modes. The carbonyl stretching modes consists of two major bands and according the study of Falk et al.[41] the higher wavenumber band is due to the stretching of the C_2-O_{11} (isolated carbonyl) while the lower wavenumber band is due to the stretching of the C_6-O_{13} (conjugated carbonyl). However, our earlier report[12] is in good agreement with Pavel et al.[42] and Nyquist and Feidler[43] experimental results (observed in solutions and condensed phase), in which the two C=O groups couple into an in phase C=O stretching vibration and an out of phase stretching vibrations. The out of phase $(C=O)_2$ stretching mode is observed at a lower frequency while the in-phase mode is observed at higher frequency.[12] Changes in the harmonic wave numbers of NH and carbonyl stretching modes due to different conformations of caffeine were found to be about 2-3.cm^{-1}, however symmetric CH_3 stretching vibrations were appreciably changed by 7 cm^{-1}. The harmonic wave numbers of selected stretching modes for all the conformers of caffeine are given in Table S4 of the ESI. The rotational constants and zero point vibrational energy of all the conformers of caffeine are listed in supplementary Table S 5. The values of rotational constants of Table S5 allow to classify the rotamers of caffeine as belonging to different families. Conformers belonging to the same family have similar mass distributions so their rotational constants are very similar. The values of rotational constants for the six conformers are seems to comparable , however classification can be concluded after comparison of these values with those observed experimentally. The relative energies, rotational constants, dipole moments and harmonic frequencies of the six most stable conformers remain for future experimental verification.

3.2. Vertical excitation energies of caffeine

The electronic absorption spectra of caffeine dissolved in water/acetonitrile are composed of broad overlapping bands located between 4.2 and 6.2 eV.[9,12,44] The electronic absorption spectrum computed in the present work at B3LYP-TDDFT/6-311++G(d,p) level is in remarkable agreement with the experiment concerning both positions and intensities of absorptions. Table 2 shows the TDDFT vertical excitation energies of the low lying singlet excited states of caffeine rotamers calculated at B3LYP-optimized S_0 geometries. For comparison with the TDDFT results, the CC2 vertical excitation energies of the lowest energy conformer A of caffeine calculated at CC2-optimized S_0 geometry are also given in Table 2. The observed UV-absorption peaks[9] are analyzed and assigned well by TD-DFT /CC2 vertical electronic excitation energies (VEE), and molecular orbital calculations of caffeine.[40] B3LYP-TDDFT and CC2 VEE for the optically bright lowest singlet excited state predicted at 4.51- 4.60 eV and 4.58 eV respectively (with an oscillator strength ~ 0.14), are found consistent with the longer wavelength absorption peak of caffeine at 4.54 eV.[9,12] The highest occupied molecular orbital (HOMO) of caffeine is a π-orbital. Its electron density is localized mainly on the C_4-C_5 fragment. The lowest singlet excited state is of ππ* character dominated by single configuration corresponding to HOMO → LUMO (0.69) excitation. Reported photoelectron spectra[40,45] and NBO calculations also show that the HOMO in caffeine molecule is formed mainly from $\pi_{C4\text{-}C5}$ bonding orbital, and shape of the HOMO of hypoxanthine, xanthine and caffeine were found very similar.[40] The energy gap between HOMO and LUMO of the most stable conformer A was found to be 5.09 eV at B3LYP/TZVP and DFT-D/ TZVP levels of theory. The dipole moment, 2.71 D, of the S_1 state is predicted lower than that of the ground state dipole moment, 3.78 D(at CC2/TZVP level), which indicates that position of S_0-S_1 band may be blue-shifted in polar solvents. Recently observed electronic absorption spectra of caffeine[9] revealed a blue shift of ~ 1 nm upon changing solvent from acetonitrile to water. Additionally it was found that caffeine relaxes to the electronic ground state from the excited state $S_1(^1\pi\pi^*)$ in only 0.48 and 1.3 picoseconds, in water and acetonitrile solutions respectively.[9] However resonant two photon ionization (R2PI) and UV-UV double resonance spectroscopy performed for the bare caffeine in the gas phase[8,11] over a wider energy range 4.12 - 4.96 eV, were reported sharp and vibrationally resolved.[8,11]This indicates long lived excited state (S_1) of caffeine. Kim et al.[11] divided their experimentally observed R2PI spectrum of caffeine into three separate regions labeled A, B and C, where the narrow region A contains lowest energy band with medium intensity, significant region B contains a group of many bands with stronger intensity and region C involve only weak and broad features. They have assigned the 0-0 band in the region A at 35246 cm^{-1}(4.37 eV) which is significantly less intense than the bands observed in region B, however the corresponding 0-0 band of theophylline (and xanthine itself) has very high intensity.[7,8] The difference between the structure of caffeine and theophylline is only a substitution of the CH_3 moiety with hydrogen at the N(7) position. It seems from earlier report[40] that HOMO in caffeine and theophylline molecules are formed mainly from the $\pi_{C4\text{-}C5}$ bonding orbital. Calculated HOMO and LUMO of caffeine and theophylline molecules are given in Fig. S1 of the ESI. The energy gap between HOMO and LUMO of theophylline was found to be 5.096 eV at B3LYP/TZVP level, which is almost similar to the value (5.090 eV) obtained for the conformer A of caffeine at the same level of theory. Additionally, the gas phase TDDFT VEE of the lowest excited $S_1(^1\pi\pi^*)$ state of the conformer A of caffeine was found (4.601 eV) very close to the value obtained for theophylline (4.609 eV). Reported ADC gas phase VEE[10] for this S_0-S_1 excitation of caffeine and theophylline are also found to be 4.825 and 4.833 eV respectively and the difference in the above excitation energy is only 0.008 eV (64.55 cm^{-1}) as found in our TDDFT calculations. Notably the assigned 0-0 band of experimentally observed R2PI spectra of caffeine[11] is about 430 cm^{-1} lower than that of theophylline.[8,11] Also it was reported[11] that the 0-0 band is significantly less intense than many of the other bands in region B, however the intense bands in region B are very close in energy to 0-0 band of theophylline.[8,11] Therefore it seems that the assignment of 0-0 band of experimentally observed R2PI spectra[11] should be reanalyzed since authors have no appropriate explanation for the assignment of low intensity 0-0 band for this optically bright $^1\pi\pi^*$ transition of caffeine in contrast to that of theophylline. As seen in Table 2 that the S_0-S_1 VEE of the six most stable conformers of caffeine (A,B,C,D,E and F) decreases towards the decreasing order of the stability. These conformers can be divided into two subsets (conformers A,B and C and conformers D,E and F) separated from one another significantly by ~ 590 cm^{-1}. VEE for S_1 ($^1\pi\pi^*$) state of conformer F was found 4.513 eV (at TD-B3LYP/6-311++G(d,p) level) is separated from that of the conformer A by 859 cm^{-1} and this shift is higher than the energy difference between 0-0 band in the region A and strong bands observed in the region B of the R2PI spectra.[11] Therefore it is possible that the assignment of 0-0 band in R2PI spectrum reported by Kim et al[11] may correspond to the origin of low energy conformers D, E and F and the intense band observed in the region B may involve the origin of 0-0 band of conformer A, B and C. So we can predict that the R2PI spectra of caffeine may consist of overlapping bands of different conformers and at this stage it is difficult to suggest a correct reassignment for the 0-0 band of S_0-S_1 transition ($^1\pi\pi^*$). Present study exemplifies the need for cross-checked experimental approaches, namely the IR/UV depletion spectroscopy, resonant ion dip IR spectroscopy and fluorescence -dip IR spectroscopy of the caffeine and the other methylated xanthines, to reach a global and consistent assignment.

The recent UV/vis spectra of aqueous/acetonitrile solutions of caffeine[9] exhibit two strong band systems in addition to a weak broad band at the shoulder of the high energy band. The energy of the observed peaks in the UV/vis spectra[9] in a acetonitrile solution obtained from authors are given in Table 2. The low energy band (245-295 nm) is due to two electronic transitions S_0-S_1 and S_0-S_2, predicted at about 4.6 and 4.95 eV respectively for the conformer A. The S_0-S_2 excitation energy (ascribed to $^1n\pi^*$ state) is dominated by H-1→L(0.67) transition. The weak broad band (at the shoulder of high energy band) observed between 5.25-5.35 eV, is attributed to S_0-S_3 and S_0-S_4 transitions predicted at about 5.18 and 5.60 eV respectively. As seen in Table 2, these two excitation energies may correspond to $^1\pi\pi^*$ and $^1\pi\sigma^*$ states which are dominated by H→L+1 (0.69) and H→L+2 (0.69) transitions respectively. Further the $^1\pi\pi^*$ states, predicted at 5.90, 5.97 and 6.19 eV at the same level of theory, are dominated by H→L+3 (0.70), H- 2→L (0.42) and H-3→L (0.59) respectively. These three electronic transitions show red shift in the aqueous solutions and correspond to very intense absorption peaks of the high energy band (200-225 nm)[9] observed at 5.95, 6.05 and 6.12 eV respectively (See Table 2). In this energy range at least five electronic transitions have been predicted by TDDFT calculations, however rest two VEE have very low oscillator strength. Our assignment has many similarities to the assignment of the excited states of Xanthine[6] and Purine.[46] Likewise, in purine[46], we may assign the excited $^1\pi\pi^*$ states, corresponds to VEE at 5.97 and 6.19 eV as Platt's ^{1}Bb and ^{1}Ba states respectively. The lowest $^1\pi\pi^*$ and $^1n\pi^*$ excitation energies of all the six conformers of caffeine at CAM-B3LYP-TDDFT/6-311++G(d,p) level are found higher than the corresponding B3LYP-TDDFT/6-311++G(d,p) energies approximately by 0.34 and 0.40 eV, respectively (See Table 2).

3.2. Optimized geometries of hydrated clusters of caffeine.

For hydration, there are three main sites in caffeine to which a water molecule can bind strongly: (1) the isolated carbonyl group (2) the conjugated carbonyl and (3) the N_9 atom. Figure 2 shows the optimized ground state structures of five lowest energy conformers of caff$_1$-$(H_2O)_1$ clusters, two O_1-bonded (involving isolated carbonyl), two O_2-bonded (involving conjugated carbonyl) and one N-bonded cluster. In each complex, the input geometry of the most stable conformer A of bare caffeine was used for the complexation with a water. These complexes are assigned as O_1-A(I), O_1-B (II), O_2-A (III), O_2-B (IV) and N bonded (V), according to the order of their stability, as shown in Fig 2. The relative energies, dipole moments and binding energies (with basis set superposition error correction) of the five lowest energy caff$_1$-$(H_2O)_1$ complexes, at MP2 and DFT (M06, B3LYP and X3LYP) levels employing the basis set 6-311++G(d,p), are listed in Table 3 and the corresponding structural parameters at MP2/6-311++G(d,p) level are given in Table S6 of the ESI. MP2 binding energies of caffeine complexes are found significantly different in comparison to corresponding DFT values. It is clear from the relative and binding energies of all five conformers that H-bonding at conjugated carbonyl site is strongest, therefore C_6=O bond is predicted as the most favorable site for hydration in caffeine. Report on the structural investigations on theophylline complexes[47] indicated that the oxygen of C_6=O bond participates in the formation of very strong intermolecular hydrogen bonding with hydrogen atom of water molecule. This interaction has apparently, an important stabilizing effect on the full structure. Similar effect is also expected in caffeine. The NBO calculations at the MP2/6-311++G(d,p) level of theory led to high negative charge densities O_{11}, O_{13}, N_1 and N_9 atoms in the O_1-bonded , O_2-bonded and N-bonded complexes (See Table S7 of the ESI). One can note that the negative charge densities on the O_{11}, and O_{13} atoms are very large in the O_2 and O_1 bonded complexes respectively. We have also computed the optimized structures and binding energies of the lowest energy caff$_1$-$(H_2O)_2$ clusters. Figure 3 shows the optimized ground state structures of the two lowest energy conformers and Figure S2 of the ESI shows the optimized structures five lowest energy conformers of caff$_1$-$(H_2O)_2$ cluster. The binding energies with BSSE correction of the two most stable conformers I and

II of caff$_1$-(H$_2$O)$_2$ cluster at the B3LYP/6-311++G(d,p) level of theory is found to 54.70 and 54.66 KJ/Mol respectively, which is double of the binding energy of the caff$_1$-(H$_2$O)$_1$ clusters.

Kim et al[11] reported only three lowest energy conformers of caff$_1$-(H$_2$O)$_1$ complexes (calculated at B3LYP/6-311++G(d,p) level), and they did not determine the optimized structure of the most stable and second most stable O$_1$-bonded clusters, involving conjugated carbonyl. Present work added the significant contribution to the knowledge about the two most lowest energy conformers of monohydrated caffeine in the isolated form. Furthermore it is important to characterize the strength of H-bonding at the two carbonyl sites with carbonyl stretching frequency shift and complex formation mechanism of the O-bonded clusters. Hydrogen bonds are able to control and direct the structures of bio-molecules because they are sufficiently strong and directional due to their noticeable electrostatic nature. In describing chemical properties of purines and their derivatives, the role of H-bonding formation and its effect on the π-electron delocalization in each particular ring and in the molecule as a whole seems of crucial importance.[16] H-bonding at the carbonyl sites of the O$_1$ and O$_2$ bonded caffeine-water complexes induce different dihedral angles between water and caffeine modifying their molecular structure with slight change in structural parameters (See Table S6 & S8 of the ESI). Consequently, variation in the dihedral angles that distort the conformation of the complex can affect its π-electron conjugation length. Interestingly, DFT calculations at B3LYP/TZVP level predicts slight decrease in the energy gap between HOMO and LUMO for the O$_1$ bonded complex while a small increase for that of the O$_2$ bonded complex, suggesting an increase and decrease in the conjugation length of the complexes respectively.

The two C=O groups in caffeine is coupled into an in-phase (C=O)$_2$ stretching vibration and an out of phase (C=O)$_2$ stretching vibration modes and the out of phase (C=O)$_2$ stretching mode is observed at a lower frequency than the in-phase mode.[12] Nevertheless, it seems that the conjugated character of the C$_6$-O$_{13}$ carbonyl, whose stretching mode strongly mixes with the C=C stretching mode to give rise out of phase (lower frequency) C=O stretching band. It was reported that the valence bond electron delocalization through the central N$_1$ atom favors lower energy for the coupled out of phase (C=O)$_2$ stretching mode.[43] Present computation predicts that due to formation of hydrogen bond, the out of phase (C=O)$_2$ stretching mode of the O-bonded caff$_1$-(H$_2$O)$_1$ clusters , is red shifted significantly (> 10 cm^{-1}) whereas the in-phase stretching mode shows a relatively small red shift (See Table S9 of ESI). Thus we may expect that the interaction of water at carbonyl site has apparently an important stabilizing effect on the full structure of caffeine. As seen in Table 3, the most stable O_1-bonded hydrated complexes involving conjugated carbonyl posses low values of dipole moment in the ground state whereas complexes involving isolated carbonyl as well as N-bonded complexes have corresponding high dipole moment values. The electronic charges from NBO calculations are found to be -0.778 at O_{11} for the isomers III and -0.773 at O_{13} atom for the isomers I, indicating relatively less polarity at conjugated carbonyl site (See Table S7). Interaction of water at conjugated carbonyl site produces a decrease in the overall charge separation which results in unusual decrease in the dipole moment of the complex I and II.

Nyquist and Fiedler[43] had discussed the interaction of caffeine with $CHCl_3$ and CCl_4 solvent in terms of carbonyl frequency shift and concluded that at lower mole percentage of solvent, complexes involving (only) isolated carbonyl is possible, however at higher mole percentage complexes involving conjugated carbonyl is possible. Infact the isolated carbonyl group for caffeine is expected to be slightly more basic than the conjugated carbonyl group, since the isolated carbonyl group is joined to two $N\text{-}CH_3$ groups while the conjugated carbonyl group is joined to one $N\text{-}CH_3$ group and to an sp^2 carbon atom.[42,43] A hydrogen-bonded complex between the water proton and the free(lone) pair of electron on the oxygen atom of the isolated carbonyl group of caffeine would then be expected to be formed before forming a complex with the free pair of electrons on the oxygen atom of the conjugated carbonyl group. Therefore, we would expect that at low molar concentration , formation of caffeine clusters (III) and (IV) are more probable in comparison to (I) and (II).

3.3. Electronic spectra and Vertical Excitation Energies of caffeine-water complexes.

We have performed calculations for the lowest lying excited state of all the five isomers of monohydrated caffeine by TDDFT method in order to estimate the spectral shift caused by micro-hydration as well as by macro-hydration. The TDDFT (B3LYP) and TDDFT (CAM-B3LYP) vertical excitation energies (VEE) to the lowest S_1 ($^1\pi\pi^*$) state, followed by S_2 ($^1n\pi^*$) state for $caff_1\text{-}(H2O)_1$ complexes are listed in Table 4. It was clear from the Table 4 that the VEE to the lowest S_1 ($^1\pi\pi^*$) state for the O_1-bonded clusters of caffeine involving conjugated carbonyl were red shifted by 363 and 444 cm^{-1} for O_1-A and O_1-B respectively. The VEE to the same state for N-bonded cluster is also red shifted slightly by 122 cm^{-1} whereas the VEE to the S_1 ($^1\pi\pi^*$) state for the O_2-bonded clusters of caffeine involving isolated carbonyl was significantly blue shifted by 444 and 549 cm^{-1} for O_2-A and O_2-B respectively. TDDFT (CAM-B3LYP) VEE shows similar trend for the micro-hydration with a slight change in the quantity of the shift as listed in Table 4. Kim et al[11] also reported a blue shift for O_2-bonded clusters of caffeine by 370 and 540 cm^{-1} at the same level of theory (TDDFT-B3LYP) for O_2-A and O_2-B which complemented the blue shifting bands observed in the electronic spectra of monohydrated clusters of caffeine, recorded in supersonic jets, using R2PI spectroscopy.[11] The effect of hydration on S_1 ($^1\pi\pi^*$) excited state due to bulk water environment was also performed by a combination of polarizable continuum solvent model (PCM)[32] and conductor like screening model (COSMO)[33], which also shows a blue shift in accordance with the result of electronic absorption spectra in aqueous solution.[9] The calculated VEE in bulk water environment are given in Table 4. Infact, hydration of a carbonyl containing heterocyclic organic molecule tends to cause a little redshift for a π-π* transition and an appreciable blue shift for n-π* transition.[48] However, neither the origin of this anomalous blue shift was explained nor any relevant reference was quoted in the support of such a blue shift (for a π-π* transition) in the earlier experimental work.[11] For heterocyclic compounds the conjugation generally results in bathochromic (red) and hypsochromic (blue) shifts in electronic absorption for a π-π* and n-π* transition respectively. A comparison of the π-π* energy gap in a series of compounds shows that longer the conjugated system, longer is the wavelength of the absorption maximum which is in consonance to the predicted red shift of π-π* transition for O_1-bonded caffeine monohydrates involving conjugated carbonyl. However in the case of smaller (or non) conjugated system, π-π* transition may exhibit opposite behavior, as reported recently by Boni et al.[43] Therefore, a blue shift for a π-π* transition is attributed to the reduction in the π-electron conjugation length[49] of the O_2-bonded caffeine monohydrates (involving isolated carbonyl) in contrast to the O_1-bonded monohydrates. The change in the electron delocalization length through the O_1 & O_2 bonded caffeine clusters is revealed by the change in oscillator strength. As seen in Table 2, oscillator strength increases in O_1 bonded caffeine clusters, whereas it decreases in O_2 bonded caffeine clusters. Additionally, it was mentioned in section 3.2 that the dipole moment of S_1 ($^1\pi\pi^*$) state, 2.7096D, is lower than that of the ground state dipole moment, 3.78 D, therefore position of S_1 state may be blue-shifted in polar solvents. The TDDFT (B3LYP) vertical excitation energies (VEE) to the lowest S_1 ($^1\pi\pi^*$) state, followed by S_2 ($^1n\pi^*$) state for caff$_1$-(H2O)$_2$ complexes are listed in Table 5. Interestingly, the VEE to the lowest S_1 ($^1\pi\pi^*$) state for the two lowest energy O-bonded caff$_1$-(H2O)$_2$ clusters show no spectral shift after the complexation.

It is clear from the above that the O_1-bonded complexes of caffeine involving conjugated carbonyl are the most stable monohydrates of caffeine. Surprisingly, the lowest energy O_1-bonded clusters of caffeine, including its N-bonded cluster, are missing in the R2PI spectrum.[11] In the R2PI experiment only blue shifted bands of O_2-bonded $caff_1$-$(H2O)_1$ clusters were detected. The missing of the most stable O_1-bonded clusters in the R2PI spectra can be explained by the assumption that water at conjugated carbonyl alters the relaxation profile by opening additional relaxation channels [1,2,9,10,44] for ultrafast decay to the ground state. The fast radiation less decay process renders the excited state lifetime of the complexes involving conjugated carbonyl too short to not allow the detection of an R2PI signal. Additionally, O_2-bonded caffeine monohydrates involving isolated carbonyl are expected to be formed before forming a O_1-bonded monohydrate involving the conjugated carbonyl group because the isolated carbonyl group for caffeine is expected to be more basic than the conjugated carbonyl group, as discussed earlier in section 3.3. It should be noted here that the intensity of the peaks of the R2PI mass spectra for $caff_1$-$(H2O)_m$ complexes obtained by nanosecond laser is rather weak, however much more intense peaks were observed by using a femtosecond laser for these clusters.[11] Femtosecond transient absorption spectra of methyl xanthines[9] revealed that in water these species relax to the ground state on subpicosecond time scale. Lifetimes of single DNA bases as well as ribonucleosides obtained by transient absorption or fluorescence measurements in aqueous solution are subpicosecond.[50] Thus the caffeine may have excited state lifetimes that rival those of adenine and guanine. The high photo stability of nucleic acid bases is consistent with the efficient nonradiative deactivation of O_1-bonded monohydrates of caffeine .

4. Conclusion

Present work reported the six stable ground state structures of neutral caffeine at MP2 level as well as at various DFT levels for the first time. Due to expected overlapping bands in R2PI spectra, present study exemplifies the need for cross-checked experimental approaches, namely the IR/UV depletion spectroscopy, resonant ion dip IR spectroscopy and fluorescence-dip IR spectroscopy studies of the isolated caffeine and the other methylated xanthines as well as their hydrated clusters, to reach a global and consistent assignment. Five lowest energy $caff_1$-$(H_2O)_1$ clusters are characterized the first time at MP2 level. Due to formation of hydrogen bond, the out of phase $(C{=}O)_2$ stretching mode of the O-bonded $caff_1$-$(H_2O)_1$ clusters is red shifted significantly (> 10 cm^{-1}) whereas the in-phase stretching mode shows a relatively small red shift. Hydrogen bonding at conjugated carbonyl site is found strongest, therefore C_6=O bond is predicted as the most favorable site for hydration in caffeine Theoretical electronic spectra (VEE) of free and hydrated complexes of neutral caffeine provide most useful diagnostic of their different conformations. They provide characteristic 'spectroscopic signatures' which can reflect differences in the nature of hydrogen-bonded interactions at different carbonyl sites. The observed and calculated blue shift for lowest $^1\pi$-π^* transition is attributed to the reduction in the π-electron conjugation length of the O_2-bonded caffeine monohydrates involving isolated carbonyl in contrast to the O_1-bonded monohydrates. Water can alter the relaxation profile by opening additional relaxation channel for the ultrafast decay (from the excited state S_1) to the ground state, which may responsible for the missing of the red shifting band in R2PI spectra of caffeine. We find that M06-2X and DFT-D methods give energy values more near to MP2 in comparison to other DFT functionals and can perform much better by employing the more higher basis sets. The CC2 and B3LYP-TDDFT approach gives the more accurate VEE for caffeine in comparison to CAM-B3LYP-TDDFT. .

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Supporting Information Available:

The structural parameters, selected harmonic wavenumber and rotational constants of all four conformers caffeine at MP2/6-311++G(d,p) level are submitted here as a supplementary information which is available in the online version of the paper. The material is available free of charge online at http://pubs.acs.org

References

1. C. E.Crespo-Hernandez; B.Cohen; P.M.Hare and B.Kohler, *Chem. Rev.,* 2004, **104** , 1977-2020.
2. E.Nir, K.Kleinermanns, and M.S. de Vries, *Nature,* 2000, **408**, 949-951.
3. M.Mons, I.Dimicoli, F. Piuzzi, B.Tardivel, M. Elhanine, *J. Phys. Chem. A* 2002, **106** , 5088–5094**.**
4. M.K. Shukla and J.Leszczynski, *J.Phys.Chem. B* 2005, **109**, 17333-339.
5. M.S. de Varies and P.Hobza , *Annu.Rev.Phys.Chem.*,2007, **58**, 585-612.
6. S. Yamazaki, A.L. Sobolewski and W. Domcke, *Phys. Chem. Chem. Phys*., 2009, **11**, 10165-10174.
7. M.P.Callahan, B.Crews, A.Abo-Riziq, L. Grace, M. S.de Varies, Z.Gengeliczki, T. M.Holmes,and G.A. Hill , *Phys. Chem. Chem. Phys.,* 2007, **9**, 4587-4591.
8. M.P.Callahan, Z.Gengeliczki, N.Svadlenak, H.Valdes, P.Hobza and M. S. de Varies, *Phys. Chem. Chem. Phys.*,2008, **10**, 2819-2826.
9. J. Chen and B.Kohler, *Phys. Chem. Chem. Phys.,* 2012, **14,** 10677-10682.
10. D.Nachtigallova, A.J.A.Aquino, S.Horn and H.Lischka , *Photochem. Photobiol. Sci.,* 2013, **12,** 1496-1508.
11. D. Kim, H. M. Kim, K. Y.Yang, S. K. Kim and N. J. Kim, *J.Chem.Phys.*,2008, **128**,134310-16.
12. S.K.Srivastava and V. B. Singh , *Spectrochim. Acta A,* 2013, **115,** 45-50.
13. C. F. Brice, A. P. Smith, *Int. J. Food Sci. Nutr.,*2002, **53,** 55-64.
14. K.Rothwell, *Nature*, 1974**, 252**, 69-70.
15. M.Ataelahi and R. Omidyan, *J.Phys. Chem.* 2013, **117**, 12842-12850.
16. O.A.Stasuk, H.Szatylowicz and T.M.Krygowski, *J.Org.Chem.,*2012, **77,** 4035-4045**.**
17. A.D. Becke, *Phys. Rev.* A, 1988, **38,** 3098-3100.
18. C.Lee, W. Yang,; R.G. Parr, *Phys. Rev.* B, 1988, **37,** 785-789.
19. A.D. Becke, *J. Chem. Phys.* 1993, **98,** 5648-5652.
20. C.Moller, M.S.Plesset, *Phys. Rev.*,1934, **46,** 618-622.
21. C.Hattig, and F. Weigned,. *J.Chem. Phys.* 2000**, 113**, 5154.
22. E.Runge and E.K.U. Gross, *Phys. Rev. Lett.* 1984, **52,** 997-1000.
23. M.J. Frisch et al, Gaussian 09, Revision A.02, Gaussian, Inc., (Wallinford CT, 2009).
24. TURBOMOLE V6.4; Turbomole Gmbh: (Karlsruhe,Germany,2012).

25. J.P.Perdew,and Y.Wang, *Phys. Rev. B,* 1992, **45**, 13244 -13249.
26. X.Xu, and W. A. Goddard III**,** *PNAS,* 2004, **9,** 2673-2677.
27. Y.Zhao and D.G. Truhlar, *Ther. Chem.Acc.* 2008, **120**,215-241.
28. E.G.Hohenstein, T.C.Samual, C.D. Sherrill., *J.Chem. Theory Comput.* 2008,**4**, 1996-2000.
29. G.Grimme, J.Antony, S.Ehrlich, and H. A Krieg,. *J.Chem.Phys.*, 2010, **132**, 154104- .
30. T.Yanai, D.P. Tew, and N.C. Handy, *Chem.Phys. Lett.* 2004, **393**, 51-57.
31.A. charaf-Eddin, A.Planchat, B.Mennucci, C.Adamo and D.Jacquemin, *J.Chem. Theory Comput.* 2013,**9**, 2749-2760.
32. Y.Tawada, T. Tsuneda, S.Yanagisawa, T.Yanai, K. A Hirao, *J.Chem.Phys.,* 2004, **120**, 8425-8433.
33. J.Tomasi, B.Mennucci**,** E. Cances, *J.Mol.Struct. (Theochem)*, 1999, **464** ,211-226.
34. D.Jacquemin, S. Chibani, B.Le Guennic and B. Mennucci, *J.Phys.Chem. A* 2014, **118**, 5343-5348.
35. D.Mafti, G. Zbancioc, I.Humelnicu and I.Mangalagiu,. *J.Phys.Chem. A* 2013, **117,** 3165-3175
36. A. Klamt and G. Schüürmann, *J. Chem. Soc. Perkin Trans*., 1993, **2** , 79
37. I. Tunon, D. Rinaldi, M. F. Ruiz-Lopez and J. L. Rivail, *J.Phys.Chem.* 1995, **99,** 3798-3805.
38. E.F.da Silva, H.F. Svendsen and K.M.Merz, *J.Phys.Chem. A* 2009, **113,** 6404-6409.
39. S.F.Boys, F. Bernardi, *Mol. Phys* 1970, **19**, 553-566**.**
40. H. Farrokhpour and F. Fathi, *J. Comp. Chem.,* 2011, **32**, 2479-2491.
41. M. Falk, M. Gil, N. Iza, *Can. J. Chem*., 1990, **68**, 1293–1299.
42. I. Pavel, A. Szeghalmi, D. Moigno, S. Cinta, W. Kiefer, *Biopolymers, 2003,* **72,** 25-37.
43. R.A.Nyquist, S.L.Fiedler, *vibrational spectroscopy* 1995, **8**, 365-386.
44. M. K.Shukla and P.C. Mishra, *J.Mol.Struct.*,1994, **324**, 241-249.
45. D.Ajo, I.Frgala, G.Granozzi and E. Tondello, Spectrochimica Acta A, 1978, **34**, 1235-1238
46. A.C.Borin, L.Serrano-Andres, M.P Fulscher, B.O. Roos, J.Phys. Chem.A, 1999, **103**,1838-1845.
47. S. Tarulli and E.J. Baran, *J. Raman Spectroscopy* 1993, **24**, 139-141.
48. G.J. Brealy, and M. J. Kasha, Am. Chem. Soc. 1955, **77**, 4462-4468.
49. L.De Boni, C. Taro, S.C. Zillo, C.R. Mendonca and F.E. Hernandez, *Chem. Phys. Lett.,* 2010, **487**,153-324.
50. C. Canuel, M. Mons, F. Piuzzi, ,B. Tardivel, I. Dimicoli, and M. Elhanine , *J.Chem.Phys.*,2005, **122**, 074316:1-6.

Table 1 Calculated relative energies (KJ/Mole) of caffeine conformers in the gas phase

Caffeine Conformers / Rotamers	MP2 Value	DFT Values				
		M06	M06-2X	X3LYP	B3PW91	B3LYP
Conformer A	**0.0**	0.0	0.0	0.0	0.0	0.0
Conformer B	**1.14**	0.47	1.09	0.69	0.61	0.77
Conformer C	**2.91**	1.87	2.44	1.94	1.91	2.01
Conformer D	**3.43**	2.55	2.88	1.78)	1.55	1.87
Conformer E	**4.44**	2.96	3.84	2.96	2.14	2.51
Conformer F	**5.99**	4.29	4.95	3.56	3.34	3.65

Table 2 Vertical excitation energies (eV) and oscillator strengths (in bracket) of the lowest energy conformers of caffeine

Approximate description of State	Wavelengths (nm) of Observed Absorption Peaks[9]	Calculated values of conformer (a) at CC2/TZVP level	Calculated values at TD-DFT/6-311++G(d,p)											
			Conformer A		**Conformer B**		**Conformer C**		**Conformer D**		**Conformer E**		**Conformer F**	
			B3LYP	*CAM-B3LYP*	B3LYP	*CAM-B3LYP*	B3LYP	*CAM-B3LYP*	B3LYP	*CAM-B3LYP*	B3LYP	*CAM-B3LYP*	B3LYP	*CAM-B3LYP*
$^1\pi\pi^*$	**4.54** **(s)**	4.582 (0.150)	**4.601** **(0.135)**	4.936 (0.172)	**4.587** **(0.137)**	4.923 (0.185)	**4.585** **(0.136)**	4.927 (0.182)	**4.528** **(0.131)**	4.8745 (0.176)	**4.515** **(0.135)**	4.863 (0.181)	**4.513** **(0.133)**	4.866 (0.179)
$^1n\pi^*$	-	4.951 (0.0)	**4.945** **(0.0)**	5.355 (0.0)	**4.941** **(0.0)**	5.338 (0.0)	**4.906** **(0.0)**	5.309 (0.0)	**4.940** **(0.0)**	5.361 (0.0)	**4.924** **(0.0)**	5.345 (0.0)	**4.905** **(0.0)**	5.317 (0.0)
$^1\pi\pi^*$	**5.25-** **(w)**	-	**5.182** **(0.001)**	5.643 (0.004)	**5.187** **(0.001)**	5.646 (0.004)	**5.181** **(0.001)**	5.644 (0.004)	**5.187** **(0.002)**	5.646 (0.004)	**5.192** **(0.002)**	5.648 (0.004)	**5.186** **(0.002)**	5.645 (0.004)
$^1\pi\sigma^*$?	**5.35** **(w)**	5.639 (0.014)	**5.559** **(0.008)**	6.030 (0.010)	**5.580** **(0.008)**	6.029 (0.017)	**5.579** **(0.018)**	6.025 (0.04)	**5.543** **(0.018)**	5.985 (0.012)	**5.540** **(0.022)**	5.982 (0.015)	**5.535** **(0.017)**	5.980 (0.012)
$^1\pi\pi^*$	**5.95** **(s)**	6.034 (0.059)	**5.894** **(0.050)**	6.367 (0.045)	**5.899** **(0.050)**	6.369 (0.046)	**5.885** **(0.008)**	6.192 (0.009)	**5.875** **(0.034)**	6.355 (0.037)	**5.878** **(0.028)**	6.354 (0.030)	**5.871** **(0.023)**	6.347 (0.028)
$^1\pi\pi^*$	**6.05** **(vs)**	6.076 (0.187)	**5.968** **(0.120)**	6.524 (0.574)	**5.970** **(0.100)**	6.521 (0.502)	**5.987** **(0.124)**	6.529 (0.556)	**5.921** **(0.115)**	6.515 (0.550)	**5.922** **(0.107)**	6.509 (0.478)	**5.943** **(0.120)**	6.514 (0.550)
$^1\pi\pi^*$	**6.12** **(vs)**	6.209 (0.109)	**6.193** **(0.381)**	6.613 (0.090)	**6.185** **(0.396)**	6.600 (0.140)	**6.186** **(0.387)**	6.6128 (0.102)	**6.158** **(0.391)**	6.576 (0.110)	**6.150** **(0.408)**	6.564 (0.198)	**6.149** **(0.396)**	6.578 (0.121)

Table 3 Relative electronic energies (kJ/mol), dipole moment (Debye) in parenthesis and binding energies (kJ/mol) of the lowest energy conformers of monohydrated caffeine calculated by DFT-B3LYP, X3LYP, M06 andMP2 employing 6-311++G(d,p) basis set.

conformer Structures	E^{B3LYP}	E^{X3LYP}	E^{M06}	E^{MP2}	$B.E^{X3LYP}$	$B.E^{M06}$	$B.E^{MP2}$
I	0.0 (1.969)	0.0 (1.974)	0.0 (1.856)	0.0 (2.475)	33.81	34.47	26.57
II	0.787 (1.932)	0.525 (1.944)	0.525 (1.826)	0.001 (2.464)	34.12	34.43	26.52
III	3.934 (4.489)	4.197 (4.462)	4.197 (4.107)	4.092 (4.726)	28.02	30.48	23.97
IV	5.246 (5.836)	5.508 (5.786)	6.033 (5.432)	4.385 (4.746)	28.01	28.90	23.52
V	5.478 (4.466)	6.033 (4.565)	6.592 (4.746)	3.844 (5.533)	26.40	28.03	23.47

Table 4 Vertical Excitation Energies (eV) and Oscillator Strengths (in bracket) of the monohydrated Clusters of Caffeine

State	Energies of Observed Absorption Peaks	**Calculated values at TD-DFT-B3LYP/6-311++G(d,p) level**						
		Bare Caffeine	Macro-hydrated PCM/COSMO^{+} Method	**Micro(mono)hydrated Caffeine**				
				Conformer **(i)**	Conformer **(ii)**	Conformer **(iii)**	Conformer **(iv)**	Conformer **(v)**
$S_1(^1\pi\pi^*)$	4.54	4.6014 (0.1348)	4.6209 (0.1882)	4.5555 (0.1456)	4.5459 (0.1489)	4.6556 (0.1320)	4.6688 (0.1338)	4.5856 (0.1408)
		4.6087^{+} (0.1759)	4.6668^{+} (0.1800)					
		_4.9360*_ _(0.1718)*_	-	_4.8755*_ _0.1940*_	_4.8684*_ _(0.1984)*_	_4.9748*_ _(0.1747)*_	_4.9938*_ _(0.1757)*_	_4.9350*_ _(0.1836)*_
$S_2(^1n\pi^*)$	-	4.9445 (0.0000)	5.1780 (0.0000)	5.0642 (0.0001)	5.0552 (0.0000)	4.9863 (0.0000)	4.9632 (0.0000)	4.9206 (0.0003)
		_5.3549*_ _(0.0000)*_	-	_5.4901*_ _0.0001*_	_5.4839*_ _(0.0000)*_	_5.3684*_ _(0.0000)*_	_5.3493*_ _(0.0000)*_	_5.338*_ _(0.0000)*_

*

***TDDFT values correspond to CAM-B3LYP Functional;** $^{+}$ TDDFT values computed at B3LYP/TZVP level

Table 5 Vertical Excitation Energies (eV) and Oscillator Strengths (in bracket) of the lowest energy caff$_1$-(H$_2$O)$_2$ clusters

States	Calculated values at TD-DFT-B3LYP/6-311++G(d,p) level		
	Bare Caffeine	Vertical excitation energies of caff$_1$-(H$_2$O)$_2$ clusters	
		Conformer **(I)**	Conformer **(II)**
S$_1$($^1\pi\pi^*$)	4.601 (0.135)	4.600 (0.144)	4.601 (0.144)
S$_2$(^{1}nπ^*)	4.945 (0.0)	5.099 (0.0)	5.0986 (0.0)

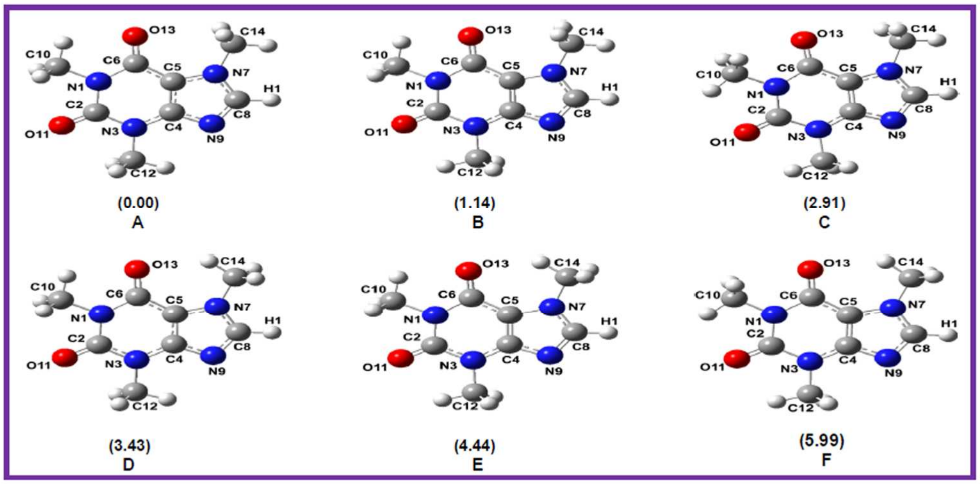


Fig.1 Optimized structures and relative energies (kJ/mol) of the lowest-energy conformers of caffeine at MP2/6-311++G(d,p) level.

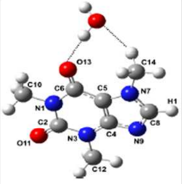
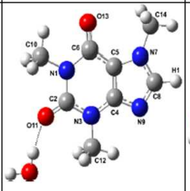
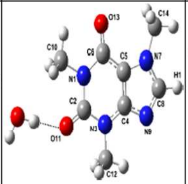
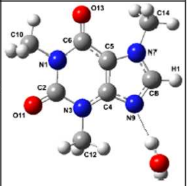
Conformer	Caff$_1$-(H$_2$O)$_1$ (O$_1$-A)	Caff$_1$-(H$_2$O)$_1$ (O$_1$-B)	Caff$_1$-(H$_2$O)$_1$ (O$_2$-A)	Caff$_1$-(H$_2$O)$_1$ (O$_2$-B)	Caff$_1$-(H$_2$O)$_1$ (N-A)
Relative energy (kJ/mol)	**0.00**	**0.69**	**4.21**	**5.60**	**5.96**
Structure	 (i)	 (ii)	 (iii)	 (iv)	 (v)

Fig.2. MP2/6-311++G(d,p) optimized geometries and relative energies of caffeine monohydrates {Caff$_1$-(H$_2$O)$_1$}. (i) & (ii) monohydrates where one of the H atoms of water interacts with O atom of conjugated carbonyl (O$_{13}$…H^{I} distance 1.8914 Å; O$_{13}$…H^{II} distance 1.8924 Å); (iii) & (iv) monohydrates where one of the H atoms of water interacts with O atom of isolated carbonyl (O$_{11}$…H^{III} distance 1.8955 Å; O$_{11}$…H^{IV} distance 1.90 Å); (v) monohydrate where one of the H atoms of water interacts with N$_9$-atom (N…H^{V} distance 1.9544 Å).

O atoms are Red, N atoms Blue, C atoms Gray and H atoms White.

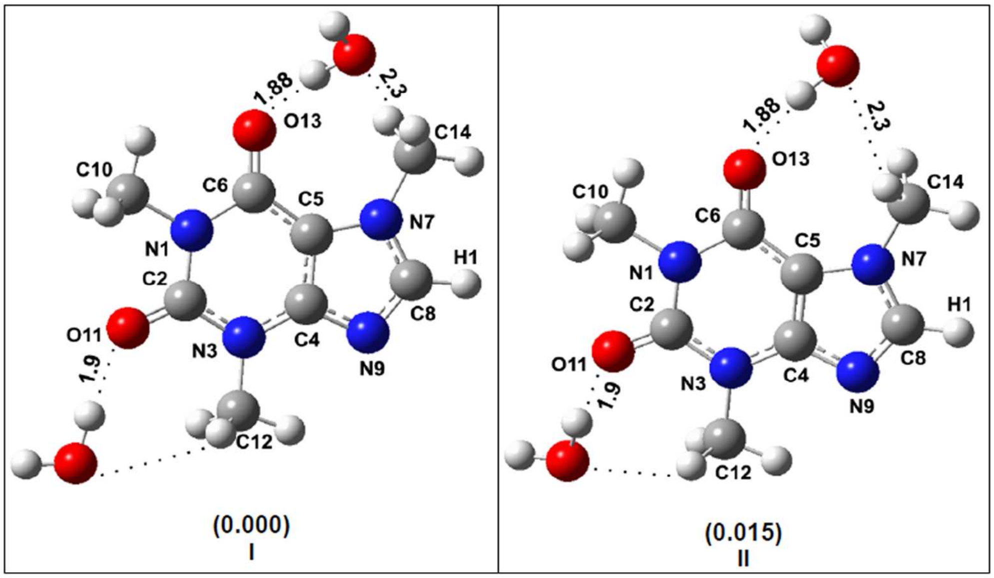
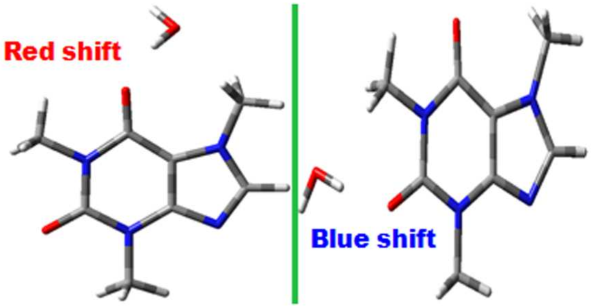


Fig. 3 Optimized structures and relative energies (kJ/mol) of the lowest-energy caff$_1$-(H$_2$O)$_2$ complexes, determined at the B3LYP/6-311++G(d,p) level.

69x46mm (600 x 600 DPI)



Blue and red shift of the VEE of the S_1 ($^1\pi\pi^*$) state of caffeine after forming complex with water at isolated and conjugated carbonyl site.