Protonation and deprotonation enthalpies of alloxan and implications for the structure and energy of its complexes with water: a computational study

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The optimized geometries, harmonic vibrational frequencies, and energies of the structures of monohydrated alloxan were computed at the DFT/ωB97X-D and B3LYP/6–311++G** level of theory. Results confirm that the monohydrate exists as a dipolar alloxan–water complex which represents a global minimum on the potential energy surface (PES). Trajectory dynamics simulations show that attempt to reorient this monohydrate, to a more favorable orientation for H-bonding, is opposed by an energy barrier of 25.07 kJ/mol. Alloxan seems to prefer acting as proton donor than proton acceptor. A marked stabilization due to the formation of N–H–OH2 bond is observed. The concerted proton donor–acceptor interaction of alloxan with one H2O molecule does not increase the stability of the alloxan–water complex. The proton affinity of the O and N atoms and the deprotonation enthalpy of the NH bond of alloxan are computed at the same level of theory. Results are compared with recent data on uracil, thymine, and cytosine. The intrinsic acidities and basicities of the four pyrimidines were discussed. Results of the present study reveal that alloxan is capable of forming stronger H-bonds and more stable cyclic complex with water; yet it is of much lower basicity than other pyrimidines.

Keywords: alloxan; gas phase acidities; dipolar alloxan–water interaction; DFT calculations; trajectory dynamics simulation; hydrogen bond

1. Introduction

Acid–base interactions involving pyrimidines are of prime importance in molecular biology (Jardetzky, Pappas, & Wade, 1963; Naik & Chikhalia, 2007; Refat, El-Korashy, Kumar, & Ahmed 2008). These interactions, which underlie the biological activity of the pyrimidine, are governed in large by its ability to form hydrogen bonds. Pyrimidine oxo derivatives are of special interest, particularly, in medicine. Thus, when studying their physiological effect on living organisms, an increased content of oxo pyrimidines was discovered in various tumor cells (Bojarski, Mokrosz, Bartoń, & Paluchowska, 1985; Patterson, Lazarow, & Levey, 1949). 5,5-Dihydroxy- 2,4,6-trioxypyrimidine (alloxan), the first known pyrimidine (Lenzen & Panten, 1988) is capable of influencing calcium, zinc, and phosphorus metabolism in organisms and of increasing the blood sugar (Lenzen, 2008). Moreover, alloxan is a toxic glucose analog, which selectively destroys insulin-producing cells in the pancreas (that is beta cells) when administered to rodents and many other animal species. Alloxan occurs in living organisms, and is the product of uric acid decomposition (Abdel-Rahman, Elrakhawy, & Iskander, 1992; Bolton, 1964; Dunn, Sheehan, & Mcleitchie, 1943; Dunitz & Schweizer, 2007; Lenzen & Munday, 1991). The high biological activity of alloxan underlies the considerable interest in understanding its structure and mode of action.

Alloxan has been widely regarded as a “problem structure.” The problem can be defined as that it contains four strong proton acceptor groups and two proton donor groups, yet its ability to form hydrogen bonds is not certain (Singh, 1965). Thus, X-ray analysis indicates the absence of such bonds (Ahmed, Soliman, & Boraei, 2004; Chandra, Nguyen, & Zeegers-Huyskens, 1998). Nevertheless, alloxan is characterized by a remarkable stability (Singh, 1965) and unusually high melting point (261 °C). Furthermore, the most stable form of alloxan is its monohydrate (Kasende & Zeegers-Huyskens, 1984) which is assumed to be a H-bonded complex.

The intrinsic acidity emerges as a common property for compounds of biological relevance; this property may be in part responsible for the natural selection of these biological molecules (Ahmed, Soliman, & Boraei, 2004; Chandra, Nguyen, & Zeegers-Huyskens, 1998) and this is likely to be the case for nucleobases (Kasende & Zeegers-Huyskens, 1984; Stewart & Harris, 1977).

Alloxan is characterized by the presence of multiple proton donor and proton acceptor sites and experimental
data on proton affinities (PA) or deprotonation enthalpies (Kasende & Zeegers-Huyskens, 1984; Stewart & Harris, 1977) are related to the most stable protonated or deprotonated forms of the examined species. For this reason, theoretical calculations can complement the experimental data. Kakkar et al. (Kakkar, Bhandari, & Gaba, 2012), in her theoretical calculations can complement the experimental data. The present study is undertaken to gain understanding on the hydrogen-bond ability of alloxan, which, like the other pyrimidines, has a great versatility in the formation of hydrogen-bond complexes because of the presence of several hydrogen-bond donor and acceptor groups. Thus, the optimized geometries, the characteristic vibrational modes, and the energies of the alloxan–water complexes are computed at B3LYP/6–311++G** level of theory. The equilibrium structure of the alloxan monohydrate is optimized using both DFT and quantum dynamics approaches. PA and deprotonation enthalpies are computed and discussed as a function of the basicity of the O atoms and the proton donor ability of the NH groups.

2. Computational details
All calculations have been carried out using the Gaussian 09 (Frisch, 2009) package of programs. The geometries of all studied compounds have been fully optimized at the DFT/B3LYP/6–311++G** level of theory (Becke, 1993; Becke, 1988; Lee, Yang, & Parr, 1988). This level of theory is chosen because this level of theory is considered in the literature as the shortest way to MP2 results (Arabi & Matta, 2011; Brovarets’ & Hovorun, 2013a, 2013b, 2014; Matta, 2010; Lozynski, Rusinska-Roszak, & Mack, 1998). Frequency calculations were performed at the same level of the theory in order to characterize the stationary points and to evaluate the zero-point energy (ZPE). The stable equilibrium structures were subjected to further confirmation and validation using other functional, namely ωB97X-D (Chai & Head-Gordon, 2008) using the same basis set. Basis set superposition error (BSSE) have been estimated using the counterpoise (CP) method of Boys and Bernardi (Boys & Bernardi, 1970). Solvent effects have been considered using the solvent continuum model, CPCM model (Foresman, Keith, Wiberg, Snoonian, & Frisch, 1996; Tapia & Goscinski, 1975).

Quantum chemical topology analysis were carried out at the quantum theory of atoms in molecules (Bader, 1990) (QTAIM) level of theory using the program AIMA11 (Keith, 2012). Delocalization indices were determined, and molecular graphs and contours and iso-surfaces of the negative of the Laplacian of the electron density were plotted, from AIMA11 (Keith, 2012).

Hyper conjugative (Reed, Curtiss, & Weinhold, 1988) interactions in the studied molecular systems have been computed using Natural Bond Orbital (NBO) theory through the calculation of the second-order perturbation energy ($E^{(2)}$); which is estimated as follows:

$$E^{(2)} = \Delta E_{p} = q_{i}(F_{ij})^{2}/\Delta \varepsilon$$

where $q_{i}$ is the donor orbital occupancy, $\Delta \varepsilon$ is the difference between the energy of an acceptor orbital ($j$) and a donor orbital ($i$), and $F_{ij}$ is the off-diagonal NBO Kohn–Sham Matrix. Throughout this work, molecular orbitals and electrostatic potential (ESP) maps were constructed using Gauss View 5.0.8 visualization program (Frisch & Denningen II et al., 2009).

Classical trajectory dynamic simulations (Steneteg, Abrikosov, Weber, & Niklasson, 2010; Sun & Hase, 2003) have been performed using the Atom Centered Density Matrix Propagation (ADMP) molecular dynamics model (Iyengar, Schlegel, & Voth, 2003; Schlegel et al., 2002).

3. Results and discussion
3.1. Alloxan monohydrate
In aqueous solutions, alloxan is assumed to exist as its monohydrate. The structure adopted for the monohydrate assumes the attachment of two OH groups to C4 of alloxan. X-ray study demonstrated that alloxan monohydrate is in the 2,5,6-triketo-4,4ʹ-dihydroxypyrimidine configuration and not the 2,4,5,6-tetraketo form with a water molecule (Singh, 1965). Scheme 1 displays this alloxan–water interaction. In fact, this 4,4ʹdihydroxy alloxan structure, which is known in the literature as the lactam form of alloxan, is the structure adopted in complex formation with transition metal ions (Naik & Chikhalla, 2007; Refat et al., 2008).

We have explored the stability of this species. The addition reaction of H2O to C4=O of alloxan has been explored. We have failed to locate this monohydrate structure on the PES as a stable stationary point. This structure seems to be a saddle point leading to the dissociation of the alloxan ring. The optimized geometric structure of the product disrupted alloxan ring is displayed in Figure 1(A). This geometry seems to be a minimum on the potential energy surface. Frequency calculations, on the full optimized geometry, show all positive eigenvalues, indicating that it represents a minimum structure on the PES.

Attempt to optimize the alloxan–water system without forcing the addition reaction has been performed. Full geometry optimization, without imposing any symmetry or geometry constrains, at the ωB97X-D/6–311++G** level of theory has converged to structure B presented in Figure 1. Again, frequency calculations show no imaginary frequency. The geometry and frequency calculations
for this monohydrate structure are given in the supplementary data. Thus, alloxan monohydrate is a dipolar interaction complex showing no hydrogen bonds. The interaction is mostly of electrostatic nature. Table 1 presents the second-order perturbation energy for the monohydrate as computed by NBO theory. Data in this table reveal that there is considerable charge transfer between donor and acceptor orbitals within the alloxan subsystem. There is no appreciable intermolecular CT interaction between alloxan and water in the complex. The stabilization of the monohydrate is thus of pure electrostatic nature. This is further confirmed by the fact that the MO’s of the monohydrate are not delocalized; they are either localized on the alloxan moiety or localized on the water subsystem. Figure 2 displays the HOMO of the monohydrate, as an example of localization of the MO’s. The ESP map of alloxan monohydrate also reflects clearly the dipolar electrostatic nature in this complex.

The PES around the minimum energy structure of the monohydrate is flat; several possible structures are close in energy and represent local minima. In order to get a much better insight into this region, we have performed a classical trajectory calculation to cover the immediate vicinity of this structure (Figure 1(B)). Starting from initial positions and velocities of the nuclei, molecular motion is propagated through time via classical mechanics, using the electronic energy and forces. These calculations provide information about how the geometry changed and distributed, and are especially useful when the region near a saddle point is relatively flat.

A DFT-based ADMP, dynamic simulation was performed starting at the fully optimized geometry of the alloxan monohydrate structure. The DFT-MD simulation was done at the oB97X-D6–31+G** level of theory using the direct inversion in the iterative subspace algorithm (Rohwedder & Schneider, 2011). The dynamics is based on the Car–Parrinello molecular dynamics model (CP) (Car & Parrinello, 1985), and is carried out in a plane wave; no pseudopotentials on hydrogen are used. In the trajectory simulation, the temperature is kept constant at 300 K and the run was submitted for 4000 points aiming to explore a conformational time domain of 400

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**Table 1.** NBO characteristics and second-order interaction energies (kcal/mol) between donor and acceptor orbitals of alloxan monohydrate.

| Center   | Charge | Configuration         | Donor no.  | Acceptor no. | ΔE  |
|----------|--------|-----------------------|------------|--------------|-----|
| N2       | −0.662 | 2S(1.29)2p(4.36)4p(0.01) | LP(1)N2    | BD*(2)C1–O12 | 65.43 |
| O9       | −0.582 | 2S(1.70)2p(4.87)3d(0.01) | LP(1)N2    | BD*(2)C3–O11 | 67.54 |
| O(water) | 0.929  | 2S(1.74)2p(5.17)      | LP(2)O12   | BD*(1)C1–2   | 33.08 |

Figure 1. (A) Disruption of the alloxan ring upon addition of water to C4=O bond. (B) the optimized geometric structure of alloxan monohydrate. The blue arrow indicates the direction of the dipole moment.
fs’s of 0.1 fs each. No Thermostats are chosen to control nuclear temperature.

The trajectory dynamics show the attempt of the water subsystem to reorient in a position more favorable for H-bonding. This is opposed by a high energy barrier and the system collapses back to the dipolar structure shown in Figure 3. The barrier height for the reorientation process is 25.07 kJ/mol and was achieved after 0.7 fs.

Results of the trajectory dynamics are summarized in Figure 1(S) of the supplementary data, whereas the geometric structure and magnitude, and direction of forces acting on atoms are displayed in Figure 3.

The trajectory calculations show an inflection in the nuclear kinetic energy profile at about 0.7 fs, this
corresponds to a maximum on the electronic kinetic energy profile. Figure 2(S) of the supplementary data displays the trajectory profiles, in the first 10 fs time domain, for some energy and geometry profiles as an example.

The geometry changes in the dipolar alloxan monohydrate structure ought to be discussed a little bit further. Thus, Figure 2(S) shows the variation of the water H–O–H angle with time. The angle tends to get smaller and reaches a minimum value of 96.5° in 5 fs. This can be attributed to the fact that water tends to maximize its dipolar interaction with alloxan by increasing the directional character of its lone pair orbital. This would change the hybridization of the oxygen atom to allow the lone pair orbital to be dominated by the p-character. There seems no tendency of the monohydrate to form H-bonds. Thus, the orientation changes of the water molecule tends to increase the C=O10–H14 distance, while the alloxan geometry suffers deformation by twisting H7 out-of-plan toward the water molecule. In both cases, the change is repulsive.

In concluding this section, it is clear that the 4,4′-dihydroxy form of alloxan monohydrate, which is assumed in the literature, cannot exist. We need to have a closer look into the X-ray analysis of (Singh, 1965). This is the work that supports the existence of 4,4′-dihydroxy structure of the monohydrate. Note that, alloxan has more than one hydrated species, namely di- and trihydrates (Lewis & Tocher, 2004). Singh did not take this fact into consideration. Furthermore, the X-ray study confirmed the existence of dipolar alloxan aggregates which complicates the spectrum and might very well lead to the unfounded conclusion of the 4,4′-dihydroxy structure. The present study confirms that the monohydrate exists as a dipolar alloxan–water complex (cf. Figure 1(B)) which represents a global minimum on the PES. Trajectory dynamics calculations show that attempt to reorient this monohydrate to a more favorable orientation for H-bonding is opposed by an energy barrier of 25 kJ/mol.

3.2. H-bonded alloxan–water complexes.

In aqueous solutions, alloxan may interact with water either as a proton donor or as a proton acceptor. As a proton donor, the four C=O groups may result in three unique alloxan–water complexes (note that O9 and O11 are identical). As proton acceptor alloxan can for only one unique complex with water. These four alloxan–water complexes are characterized by the formation of just one H-bond. However, there remains the possibility of the formation of three cyclic complexes via the formation of two H-bonds. We have investigated all different alloxan–H2O orientations where full geometry optimizations and frequency calculations have been carried out.

The most relevant of these interactions are presented in Table 2. The dipolar alloxan–water complex, suggested in the present work for the structure of alloxan monohydrate, is also included in Table 2, as complex 4, for comparison. Alloxan seems to prefer acting as proton donor. A marked stabilization due to the formation of N–H–OH2 bond is observed. The concerted proton donor–acceptor interaction of alloxan with one H2O molecule does not increase the stability of the alloxan–water complex. The effect seems additive, thus complexes 2 and 3 which are almost of the same stability show doubling of their stabilization energy upon interaction with a second water molecule. This point is of prime importance when considering the intramolecular proton transfer process in alloxan. Thus, water-assisted proton transfer may very well underlie the marked acidity observed (pKa = 6.8) for alloxan (Ahmed, Soliman, & Boraei, 2004; Chandra, Nguyen, & Zeegers-Huyskens, 1998).

Let us investigate the atomic basins for the alloxan–water complex shown in Figure 4. The intensity of overlap of the contour lines for the N–H–OH2 region is more pronounced than in the C=O–H region (Brovarets’, Yurenko, & Hororun, 2014). This is also reflected in the value of the Laplacian of the electron density at the two BCP’s, he ωB97X-D /6-31++G** optimized geometries of the six alloxan–water complexes are shown in Table 2. In the most stable of these complexes (complex 2), alloxan acts as proton donor. Complex 3 involves the formation of a closed six-membered ring water complex. In which, one water molecule accepts the acidic NH proton while donating a proton to the carbonyl oxygen of alloxan. The H-bonded alloxan–water complexes are characterized by C1 symmetry, one of the hydrogen atoms being out-of-plane of the alloxan ring. Characteristic geometrical parameters of free alloxan and its water complexes are indicated in Table S1 of the supplementary data.

The C–C distances in alloxan and the bond angles in the ring are very insensitive to complex formation and are not indicated in these tables. We can also notice that the variation in the bond distances is small yet it can be traced in the reported geometric values for C=O, N–H, and HOH bond angle. For complex 3, where the six-membered ring is formed, the ring formation forces an increase in the H–O–H angle with a consequent increase in the O12–H13 bond length which is involved in the H-bond formation. In case of complex 2, the C5=O bond length shows slight elongation.

Table 3 presents a comparison between the relevant vibrational frequencies of alloxan and its water complexes. For complexes 2 and 3, there is a considerable shift to lower frequencies of the asymmetric N–H stretching modes. This would imply the decrease in the N–H force constant upon H-bond formation. The water O–H stretching vibration also shows the same trend.
Table 2. Geometries, binding energies, and Gibb’s free energies for the alloxan–water complexes considered in the present work computed at the \( \omega B97X-D/6-311++G^{**} \) level of theory.

| Alloxan–water complex | Interaction Distance (Å) | Gibbs free energy/a.u. | Binding energy/kJ/mol Without BSSE | Binding energy/kJ/mol With BSSE |
|-----------------------|--------------------------|------------------------|-----------------------------------|---------------------------------|
| 1                     | 2.09 (O11…H13)           | −640.40886             | −20.54309                         | −18.081                         |
|                       | 2.61 (O10…H14)           |                       |                                   |                                 |
| 2                     | 1.87 (H7…O13)            | −640.41775             | −43.42041                         | −39.698                         |
|                       | 2.18 (O9…H15)            |                       |                                   |                                 |
| 3                     | 1.86 (H8…O13)            | −640.41752             | −43.51253                         | −39.592                         |
|                       | 2.16 (O12…H14)           |                       |                                   |                                 |
| 4                     | 3.25 (O9…H15)            | −640.41673             | −38.83734                         | −33.768                         |
|                       | 2.70 (C4…O13)            |                       |                                   |                                 |

(Continued)
There seems a direct correlation between the extent of stabilization of the alloxan–water complex and the magnitude of the frequency shift of the C=O groups. Thus, complex 1, for example, does not show any marked shift of $\nu_{C=O}$ whereas, complex 2 shows $\nu_{C4=O}$ shifted to lower frequencies by almost 20 cm$^{-1}$. It is worth noting that C4=O is not coupled with any other C=O vibration mode. Whereas, the other three C=O vibrations are shifted to lower frequencies as compared to $\nu_{C4=O}$. The vibration band at 1837 cm$^{-1}$ is a coupling band involving the C5=O, the N–H bending, and the H–O–H angle bending mode.

This high intensity vibration corresponds to the complex rather than any of its subsystems. This coupling vibration, which is not observed in case of complex 1 or complex 4, is displayed in Figure 5.

To complete the picture and understand a little bit more fully the behavior of the alloxan–H$_2$O complexes, let us perform dynamic trajectory simulation for the most stable of these complexes. Thus, for complex 2, an ADMP dynamic simulation was performed starting at the fully optimized geometry of the alloxan–H$_2$O complex 2 structure. The DFT-MD simulation was done at the $\omega$B97X-D/6-31 + G** level of theory. In the trajectory simulation, the temperature is kept constant at 300 K and the run was submitted for 4000 points aiming to explore a conformational time domain of 400 fs. of 0.1 fs each.

The trajectory dynamics show the attempt of the water subsystem to reorient in a position more favorable for H-bonding. After crossing a low barrier, the energy of the complex increases continually accompanying the geometry changes. The O…H hydrogen-bond length as well as the H–O–H bond angle show continuous oscillations in the meantime, the H$_2$O subsystem attempt to rotate out-of-plan of the alloxan ring, yet this is also opposed by a marked energy barrier. In conclusion, the geometric structure displayed in Table 2 for complex 2 is the global minimum structure for the H-bonded alloxan–H$_2$O complex. Results of the trajectory dynamics are displayed in Figure 3(S).

In conclusion, the interaction between alloxan and water may take place either via dipolar or H-bond interactions. Both are of comparable stabilization energies. In the less condensed more polarizable medium, in solution, the H-bonded alloxan–water complex prevails. However, the dipolar alloxan–water complex seems to be the predominant species in the solid state. This type of interaction allows for the formation of aggregates and provides a quantitative explanation for the absence of H-bonds in the X-ray experimental data of alloxan monohydrate.

The table below shows the binding energy and the Gibbs free energy for the alloxan–water complexes.

| Alloxan–water complex | Interaction Distance ($\AA$) | Gibbs free energy/au | Binding energy/kJ/mol |
|-----------------------|-----------------------------|----------------------|-----------------------|
|                        |                             |                      | Without BSSE          | With BSSE             |
|                        |                             |                      | Binding energy/kJ/mol  |                      |
| 5                      | 1.89 (H7…O13)               | −716.84525           | −86.69115             | −79.221               |
|                        | 2.15 (O9…H15)               |                      |                      |                      |
| 6                      | 1.87 (H8…O13)               | −716.84512           | −85.78788             | −77.836               |
|                        | 2.12 (O12…H14)              |                      |                      |                      |
3.3. Protonation and deprotonation of alloxan

Proton affinity \( P(A) \) is defined as the negative of the molar enthalpy change at 298.15 K (\( \Delta H^\circ \)) for the reaction \( A + H \rightarrow AH^+ \), and may be given as follows:

\[
P(A) = -\Delta E_{\text{elec}} - \Delta ZPE - \Delta E_{\text{vib}} + 5/2RT
\]  

(2)

where, \(-\Delta E_{\text{elec}}\) is the change in the electronic energy upon reaction, \(\Delta ZPE\) is the change in the zero point energies, and \(\Delta E_{\text{vib}}\) is the change in energy associated with internal vibrations of reactants and products. All values of \(\Delta E_{\text{vib}}\) are small for molecules of this size, in our case on the order of 4 kJ/mol or less. Consequently,
the differences between $E_{vib}^0$ values for the parent and protonated molecules are negligibly small compared with those for the experimental error in proton affinity measurements. For larger molecules, $E_{vib}^0$ can also be safely omitted in proton affinity calculations since the largest contributions to $E_{vib}^0$ come from the lowest frequency vibrations (e.g. torsions around single bonds) and these are for the most part not substantially affected by protonation. Thus, $\Delta E_{vib}^0$ are omitted in our calculations of PA values, so also the constant value of $5/2RT$.

The PA of the oxygen atoms and the deprotonation enthalpies of the NH bonds of alloxan have been calculated at the B3LYP density functional level of theory using the 6–311+G** basis set. Table 4 lists zero-point vibrational energies differences ($\Delta ZPE$) and the PA of all possible protonated species of alloxan. The value of ZPE is proportional to the sum of vibrational frequencies, and, therefore, it is primarily affected by larger frequencies (e.g. bond stretching and bending vibrations). The differences in ZPE for protonated and parent molecule is, therefore, not small and in our case approaches 120 kJ/mol. Protonation results in the formation of a new covalent X-H bond and also affects geometry and strength of vicinal bonds. For that reason, $\Delta ZPE$ must be accounted for in proton affinity calculations. Also, systematic over or underestimation of frequencies will bias the value of ZPE in the same direction. Note that, values of PA reported in Table 4 indicate that protonation at the N2 (or N6) is much less favorable by more than 100 kJ/mol than protonation at the O sites. Three different O protonation sites are available in alloxan.

Inspection of the net atomic charges on the oxygen atoms of alloxan reveals that O12 seems to be the most negative O site, followed by O9 and O11 and finally O10; the corresponding PA values are listed in Table 4. Indeed, the most favorable site for protonation seems to be O9 (or O11). The orientation of the O–H group toward C=O10 oxygen lone pair orbital allows for an additional stability due to H-bonding. However, the C–O10–H bond distance of 2.22 Å suggests a weak interaction. Protonation at the O10 site is of the least PA value. This seems reasonable since there is a marked withdrawal of charge from the C=O10 bond region by the two neighboring carbonyl groups. This would decrease the polarization and, hence, the tendency to attract the proton.

The calculated PA of the most basic site of alloxan (O9 or O11) 740 kJ/mol is about 100 kJ/mol lower than the experimental values for other pyrimidines (uracil (Nguyen & Chandra, 1998) PA = 860 and thymine (Russo, 1998) PA = 873 kJ/mol). The PA of the O12 atom is sensibly lower. The HOMO of alloxan is
composed of a combination of the oxygen and nitrogen in-plan lone pair orbitals, then (O9 and O11) orbitals have the largest contribution and have considerable spatial extent (cf. Figure 6). The energy of the HOMO (746.5 kJ/mol) correlates very well with the computed PA of alloxan. The difference between the PAs of the N and O atoms is quite substantial and amounts to about 100 kJ/mol. This is in line with recent ab initio calculations which have shown that amides derived from weak acids protonated preferentially at the acid residue rather than at the nitrogen with energy differences ranges between 40 and 120 kJ/mol (Bonaccorsi, Pullman, Scrocco, & Tomasi, 1972; Cho et al., 1997).

Frequencies of some selected vibrational modes of the four protonated forms of alloxan are displayed in Table 5. Positions and intensities of the N–H, and OH stretching bands seem very sensitive to the site of protonation. O10 protonated species shows the N–H stretching (sym. and asym.) at 3525 and 3520 cm⁻¹, respectively, followed by a moderate band at 3273 cm⁻¹ corresponding to the O–H stretching vibration. The O9 and O11 protonated forms show different spectra, however, the νOH now appear at a much higher frequencies of 3561 and 3750 cm⁻¹, respectively, followed by the N–H stretching bands in almost the same frequency range of 3525–3476 cm⁻¹. However, new vibration band at 1719 cm⁻¹ of high relative intensity is observed. This vibration band is a coupling band between the C=O, the O–H and the C–N bond stretching modes, and characterize the protonated species. This vibration mode is shown in Figure 7 for the case of O11 and O12 protonated forms of alloxan. Protonation causes a marked shift of

| Protonation sites | Charge | ΔE_ZPVE (kJ/mol) | PA (kJ/mol) |
|------------------|--------|-----------------|-------------|
|                  |        |                 | Gas | Solution |
| –N2              | −0.366 | 28.95           | 646.95 | 590.61 |
| –O12             | −0.286 | 29.82           | 740.55 | 677.39 |
| –O9 (O11)        | −0.248 | 32.13           | 762.32 | 677.77 |
| –O10             | −0.180 | 31.27           | 707.61 | 621.05 |

Table 4. All possible protonated forms of alloxan and their total, relative, protonation, zero-point vibrational energies in the gas phase and in water as a solvent computed at the DFT/ B3LYP/6–311++G** level of theory.
the νC=O to higher frequencies as compared with that of alloxan itself.

Classical trajectory dynamics calculations have been performed for the O12 protonation. The approach of the proton is favored by a lowering in the potential energy of 263 kJ/mol. This is achieved after 4.5 fs along the trajectory. On the other hand, an electronic kinetic energy barrier of 20.4 kJ/mol opposes this approach and appears very early along the trajectory at 0.8 fs. Along a trajectory of 100 fs, the proton oscillates back and forth.

Table 5. Comparison between some vibration modes of alloxan and the corresponding values for its protonated forms.

|                      | Alloxan (ν) (cm⁻¹) | Protonated alloxan (ν) (cm⁻¹) |
|----------------------|--------------------|--------------------------------|
|                      |                    | 1                             | 2                             | 3                             | 4                             |
| N–H                  | 3576.34            | 3499.69 (N6–H7 only)          | 3525.45                       | 3520.87 (N6–H7 only)          | 3525.26                       |
| (as.) N–H           | 3573.4             | 3413.33 (N2–H8 only)          | 3513.09                       | 3476.37 (N2–H8 only)          | 3520.05                       |
| (Η–X*)               | –                  | 3350.81                       | 3750.13                       | 3561.55                       | 3273.72                       |
|                      |                    | 3413.33 (as.)                |                                |                               |                               |
| C=O12                | 1806.55            | 1937.07                       | 1665                          | 1887.05                       | 1852.27                       |
| C=O11                | 1822.06            | 1957.02 (C=O11 only)          | 1890.55                       | 1719.58 (C=O11, N2–C3)        | 1812.01                       |
| C=O9                 | 1798.94            | 1855.62 (C=O9 only)           | 1871.95                       | 1832.51 (C=O10, C=O9)         | 1783.73                       |
| (as.)C=O11          |                    |                               | 1845.71                       | 1849.66 (as.C=O10, C=O9)      | 1679.3                        |
| C=O9                 | 1844.49            | 1842.19                       |                                |                               |                               |

*X = N,O.*

Figure 6. Selected alloxan MO’s involved in protonation/deprotonation process.

Figure 7. Coupled vibrational bands in O11 and O12 protonated form of alloxan.
between 0.8 and 1.65 Å. This is accompanied by a periodic change in the H–O–C angle and the dihedral angle controlling the twist of the proton out-of-the plane of the ring. These changes are summarized in Figure 4 (S). Some selected geometric parameters of protonated alloxan forms are listed in Table S2 of the supplementary data. Note that the O12 protonated form is more stable than the starting optimized structure by ~15 kJ/mol. This stabilization energy came on the expense of deformation of the geometry and loss of co-planarity.

Table 6 lists the deprotonation energies of the NH bonds obtained from DFT calculations. NH bonds are characterized by acidity which is sensibly lower than that of formamide (1500 kJ/mol), N-methylformamide (1510 kJ/mol) or N-methylacetamide (1514 kJ/mol) (Mizushima et al., 1950; Ventura, Rama, Turi, & Dannenberg, 1995) showing that the presence of the NH bond in between two carbonyl groups has a major effect on the acidity. Furthermore, alloxan is less acidic than other pyrimidines such as uracil (1361 kJ/mol), although the environment of the NH bond in the two compounds is almost the same. The reason for this reduced acidity is probably a strong delocalization of the electron density over the C(O)–N(H)–C(O)–N(H)–C(O) part of the molecule, with a consequent decrease of the ionic character of the N–H bond. The first highest π MO (HOMO-1) reflects nicely this delocalization. The acidity of alloxan is also reflected by its pK in water (6.3) (Ahmed, Soliman, & Boraei, 2004; Chandra, Nguyen, & Zeegers-Huyskens, 1998).

It is worth noting that the change in the ZPE values upon deprotonation is considerably reflecting a major geometry changes. Detachment of a proton causes substantial deformation of the geometry of alloxan. Thus, upon removal of H7, there is a marked shortening of the N6–C5 bond by about 7% and a considerable elongation

| Deprotonation sites | Charge | ΔE_{ZPVE} (kJ/mol) | PDE (kJ/mol) |
|--------------------|--------|------------------|--------------|
| N·H                | −0.366(N) | −69.74          | 1770.33      | 863.78 |
| 2(N·H)             | −0.366(N) | −39.90          | 1366.23      | 1795.82 |

Table 7. Binding energies* Including ZPE and BSSE corrections for the different cyclic structures some selected pyrimidines complexed with water and the PA* and PDE* values of the pyrimidine.

| Pyrimidines | BE   | PA**          | PDE             |
|-------------|------|---------------|-----------------|
| Uracil      | 32.7 | O4: 849       | N1H: 1391.0 N3H: 1447.1 |
| Thymine     | 32.2 | O4: 854.4     | N1H: 1398.1 N3H: 1450.0 |
| Cytosine    | 37.2 | N3: 955.5     | N1H: 1444.5 N7H8: 1481.8 N7H9: 1457.1 |
| Alloxan     | 37.1 | N: 646.9, O12: 740.5, O9: 792.3, O10: 707.6 | N–H: 1770.333 |

*all energy quantities are in kJ mol⁻¹; with the exception of alloxan all energy quantities are computed at the B3LYP/6-31 + G(d,p) level of theory. [10].

**for the most basic site.
of the C4–C5 bond by 15%. This is accompanied by loss of the co-planarity of the alloxan ring. Table S3 of the supplementary data summarizes the observed geometry changes accompanying the deprotonation process.

In concluding this section, it seems appropriate to compare the results obtained in this work with those computed at comparable level of theory for other pyrimidines. Table 7 presents such a comparison for the binding energies of the cyclic structure of the pyrimidine–water complexes and the computed PA and PDE values.

Inspection of Table 7 reveals that alloxan seems capable of forming stronger H-bonds and a more stable cyclic complex with water than other pyrimidines. Alloxan is of much lower basicity than other pyrimidines. Thus, although, C=O12 is in a similar molecular environment as that in the other pyrimidines, yet it seems that the effect on the basicity is not local but rather global. This explains the different PA values of uracil and thymine which have identical structure except for the methyl group which is in the γ-position to the carbonyl group. Furthermore, the intrinsic acidity of alloxan is also much lower than that for other pyrimidines. This point has been raised before and explained on the basis of the lower ionic character of the N–H bond due to delocalization of the charge density.

Conclusions

Results of the present study quantitatively indicate that alloxan monohydrate exists as a dipolar complex which explains the experimental observation of the absence of H-bonds in its X-ray structure data. On the other hand, in a less condensed more polarizable medium, the H-bonded alloxan–water complex prevails. In the present work, the PAs of the different basic sites and the deprotonation enthalpies of the NH bond of alloxan are for the first time computed at an accurate level of theory and compared with those computed for other pyrimidine bases. It is shown that intrinsic acidities and basicities can be considered as key factors for understanding the energies and vibrational properties of hydrogen bonds formed between pyrimidine bases and a water molecule.

Supplementary material

The supplementary material for this paper is available online at http://dx.doi.10.1080/07391102.2014.919238.

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