Ab initio and DFT studies on ionization of octopamine and 6-aminopenicillanic acid in aqueous solution

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The existing quantum chemical methods to accurately predict pK a values and to determine the trade-off between accuracy and computational cost have been evaluated by ab initio and density functional theory with the B3LYP functional and 6-31+G(d) basis sets and polarizable continuum solvation model. The calculated free energies for determination of pK a values, and intermolecular hydrogen bonds in aqueous solutions of octopamine and 6-aminopenicillanic acid have been computed. This study shows that there is reasonable agreement between the theoretically calculated pK a values and the experimentally determined pK a values for the acid-base reactions obtained by potentiometric and spectrophotometric methods reported in the literature.

Keywords: Theoretical chemistry, Ab initio calculations, Density functional calculations, Acid dissociation constant, 6-Aminopenicillanic acid, Octopamine

(+)-6-Aminopenicillanic acid (6-APA) is an intermediate product with special importance for the pharmaceutical industry since it is the main starting block for the preparation of numerous semisynthetic penicillins (β-lactam antibiotics) by acylation, esterification, amidation and hydroxyamidation reactions, etc., which allows new derivatives with enlarged spectrum of biological activity 1 to be obtained. Octopamine (β, 4-dihydroxyphenethylamine) is one of the most abundant biogenic amines in the nervous system of vertebrates that is closely related to norepinephrine, and has effects on the adrenergic and dopaminergic systems. It is also found naturally in numerous plants, including bitter orange and various insect tissues.2,3

It is well-known that the acidity constants are important for the development of new compounds with biological activity. For the determination of acidity constants, capillary electrophoresis4, calorimetric adsorption method, potentiometric titration method5, chromatographic methods6 and IR, NMR and UV-visible spectrometric7-10 determinations in water or in mixtures of solvents are used. In addition to experimental methods, quantum chemical methods have been developed for the calculation of pK a values based on chemical structures. Some studies dealing with the acid-base properties of compounds in aqueous solutions and in gas phase are also available11-13. Hence, reliable and accurate methods for calculating relative and/or absolute pK a values are important to have an understanding of the effective pK a values in molecules.

Computational chemistry emerges as a valuable tool for evaluating the right sequence of deprotonating. This explains the many attempts to develop reliable methods for the calculation of accurate absolute or relative pK a values6-8. Computational determination of accurate pK a values for unusual solutes such as free radicals, zwitterions and excited states remains elusive16-18. Accuracy in pK a calculations is difficult to achieve because an error of 1.36 kcal/mol in the change of free energy for deprotonation in solvent results in an error of one pK a unit.

Herein, we have studied two molecules, viz., 6-aminopenicillanic acid which is a basic penicillin structure, and, octopamine which is a biogenic amine. The main objective of this work is to calculate the acidity constants of the studied compounds by ab initio methods and compare the values with reported experimental pK a values.
Computational Methods

The optimized structures of octopamine and 6-APA were carried out using ab initio Hartree-Fock methods and the results were reoptimized with DFT at B3LYP. All the initial geometries and solvated molecules in water, considering the intermolecular hydrogen bonds of water with amino, hydroxyl and carboxyl groups, were optimized with the Gaussian 09 program packages using hybrid density functional B3LYP, the Becke’s three-parameter exchange functional12, and the Lee-Yang-Parr correlation functional13 using 6-31+G(d) basis function. For interactions in the presence of solvent molecules in an aqueous environment, an aqueous solvent cage was added to the model using the polarizable continuum model (PCM). In this method, the solvent is represented as a structureless polarizable medium characterized by its dielectric constant20.

The optimized structures including 

\[ \text{H}_2\text{L}^\text{3+}, \text{H}_2\text{L}, \text{HL}^-, \text{L}^- \text{ for octopamine and } \text{H}_2\text{L}^+, \text{HL}^- \text{ and } \text{L}^- \text{ for 6-APA and also their } n\text{-hydrated } ((\text{H}_2\text{O})_n, n = 1\text{–}4) \text{ structures have been optimized at the B3LYP/6-31+G(d) level of theory. The single-point calculations at the same level of theory have been employed for solvent effect interactions using the polarizable continuum model (PCM) of Tomasi and coworkers in the Self-Consistent Reaction Field (SCRF) model14–20.}

Finally, solvation of the species was selected by means of intermolecular hydrogen bonds (IHBs) that involve one molecule of the mentioned species and some molecules of water (see Table 1).

<table>
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<tr>
<th>Solvated species</th>
<th>( G^\text{o} \text{sol} ) (Hartree)</th>
<th>Solvated species</th>
<th>( G^\text{o} \text{sol} ) (Hartree)</th>
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Results and Discussion

Octopamine and 6-APA drugs have the tendency to lose three and two acidic hydrogen respectively. For 6-APA, a proton can be lost from either of the two groups to give different ionized species; the loss of a proton from the carboxyl group is most probable and from the ammonium group least probable. Therefore, the concept of microscopic ionization constants, \( k_1 \) and \( k_2 \), may be applied, where \( k_1 \) involving the carboxyl proton is:

\[
k_1 = \frac{[\text{H}^+][\text{NH}_3^+\text{CHRCOO}^+]}{[\text{NH}_3^+\text{CHRCOOH}]} \quad \ldots(1)
\]

And \( k_2 \) involving the ammonium proton is

\[
k_2 = \frac{[\text{H}^+][\text{NH}_2^+\text{CHRCOOH}]}{[\text{NH}_3^+\text{CHRCOOH}]} \quad \ldots(2)
\]

It can be shown that for a dibasic acid, the first ionization constant \( K_1 \) is the sum \( k_1+k_2 \) and the second ionization constant \( K_2 \) is \((k_{12}·k_2)/(k_{12}·k_{21})\), where the subscript 12 denotes the loss of proton 2 following the loss of proton 1 and subscript 21 denotes loss of proton 1 following the loss of proton 2.

The chemical interpretation of the changes is not straightforward, even though from model compounds the carboxyl proton is predicted to be the most acidic. Calculations involving the microscopic constants indicate that the first \( K \) corresponds to removal of the carboxyl proton and the second \( K \) to removal of protons from the ammonium group. However, this can be determined accurately only by NMR spectroscopy21–23.

The different models of molecules (zwitterions and unzwitterions) were investigated by the G09 program24. Considering Eqs 1 and 2, different reactions including cationic, neutral, and anionic species were tested, but some of the reactions were not considered further because the estimated error in its acidic dissociation constants was unacceptable. The models finally chosen for the studied system and the calculated values of the acidic dissociation constants for different drugs are listed in Table 2.

The acidic dissociation constant values of drugs determined using the potentiometric technique and reported in literature are used in this work. These values are listed in Table 2 along with the values calculated herein using the Tomasi method at the B3LYP/6-31+G(d) level of theory.
it is inferred that reaction (Eq. 6) and taking into account Eqs 4 and 5, Considering these facts and to provide a more satisfactory representation of the protolysis of water, the reaction has been considered as follows:

\[
5H_2O \rightleftharpoons OH^+ + H_3O^+ \quad \text{...(6)}
\]

According to the above reactions, both H\(^+\) and OH\(^-\) ions are hydrated with one water molecule. Considering \(K_w\) as the equilibrium constant of the reaction (Eq. 6) and taking into account Eqs 4 and 5, it is inferred that \(K_w = [H_2O]\)

where \([H_2O]\) is the molar concentration of water. Consequently, at 298.15 K, \(K_N\) was calculated by Eq. (7).

\[
K_N = K_w/ [H_2O] = 1.831 \times 10^{-16} \quad \text{...(7)}
\]

For disintegrate ion of five water molecules, the reactions has been shown in Eq. (8).

\[
5H_2O \rightleftharpoons OH(H_2O)\_3 + H_3O^+ \quad \text{...(8)}
\]

\[
K_M = K_w/ [H_2O] = 6.4149 \times 10^{-20}
\]

The total energies of the single and solvated drugs (octopamine and 6-APA) species (cationic, neutral, and anionic) were calculated in water at the B3LYP/6-31+G(d) level of the theory, using Tomasi’s model. Table 1 summarizes the free energies (solvation) of the species per water molecule (Hartree) as a function of the total number of solvation water molecules, (Fig. 1 and Table 1). A marked increase in the total energies (in absolute values) of ions is observed when the number of solvent molecules (water) increases.

These data show that the water, exerting its hydrogen-bond-donor (HBD) capability, forms intermolecular hydrogen bonds (IHBs) with the octopamine and 6-APA anions\(^{27}\). These hydrogen bonds have been classified as strong, moderate, and weak, according to their lengths, angles, and energies\(^{28}\).

**First ionization constant of 6-APA and octopamine**

It was observed that in alkaline solutions, 6-APA and octopamine undergo partial neutralization as follows:

For 6-APA:

\[
H_2L^+ (H_2O) + OH^- (H_2O) \rightleftharpoons HL(H_2O)_3^+ \quad K_{C1} \quad \text{...(9)}
\]
For octopamine:

\[ H_3L^+(H_2O) + \text{OH}^- (H_2O)_3 \rightleftharpoons H_2L(H_2O)_2 + 3H_2O \quad \text{K}_{d1} \]

\[ \text{...} \text{(10)} \]

In these reactions, \( H_2L^+(H_2O) \) is the 6-APA cation solvated with one water molecule, \( HL(H_2O)_3;Z \) represents 6-APA solvated with three water molecules, \( H_3L^+(H_2O) \) is the octopamine cation solvated with one water molecule and \( H_2L(H_2O)_2 \) is the neutral octopamine solvated with two water molecules.

By combining Eq. 9 with Eq. 6, and Eq. 8 with Eq. 10, we obtain Eqs 11 and 12, which define the first ionization constant of 6-APA (\( K_{al} \)) and octopamine (\( k_{b1} \)) respectively and show the solvation of the neutral 6-APA and octopamine (Eqs 11 and 12).

For 6-APA:

\[ H_2L^+(H_2O) + 3H_2O \rightleftharpoons HL(H_2O)_3;Z + H_3O^+ \quad K_{al} \]

\[ \text{...} \text{(11)} \]

For octopamine:

\[ H_3L^+(H_2O) + 2(H_2O) \rightleftharpoons H_2L(H_2O)_2 + H_3O^+ \quad K_{b1} \]

\[ \text{...} \text{(12)} \]

It is evident that

\[ K_{al} = K_{a1} \times K_N \quad \text{and} \quad K_{b1} = K_{d1} \times K_M \]

The above equations were used to determine theoretically the value of the first ionization constant of drugs in water. Table 2 summarizes the optimized values of molecular properties of the \( H_2L^+(H_2O) \) cation, \( \text{OH}^-(H_2O)_3;Z \) neutral molecule for 6-APA (Fig. 2) and \( H_3L^+(H_2O) \) cation, \( \text{OH}^+(H_2O)_3; \) anion, \( H_2L(H_2O)_2 \) neutral for octopamine (Fig. 3) obtained at the B3LYP/6-31+ G(d) level of theory with Tomasi’s method in water at 298.15 K.

It may be noted that in the formation of the neutral 6-APA solvated with three water molecules, the neutral molecules practically do not have the structure that characterizes the solvated 6-APA cation (Fig. 2 and Table 3).

Obviously, the formation of the neutral 6-APA implies that the electronic density of the O\(_{15}\) atom increase notably (in absolute value) with respect to the O\(_{15}\) atom of the 6-APA cation. Also, for octopamine it is observed that electronic density of O\(_{2}\) of the neutral molecule decreases with respect to O\(_{7}\) atom of the octopamine cation (Tables 3 and 4).

On the other hand, the theoretically obtained \( pK_{al} \) values of the 6-APA and octopamine (\( pK_{al} = 2.4419 \) and 8.985 respectively) are comparable with the respective experimental \( pK_{al} \) values\(^{29,31} \) (\( pK_{al} = 2.50 \) and 8.88).
Second ionization constant of 6-APA and octopamine

It is observed that the neutral species $\text{HL}(\text{H}_2\text{O})_3:Z$ (for 6-APA) and $\text{H}_2\text{L}(\text{H}_2\text{O})_3$ (for octopamine), undergo total neutralization as follows:

For 6-APA:

$$\text{HL}(\text{H}_2\text{O})_3:Z + \text{OH}^- \rightleftharpoons \text{L}(\text{H}_2\text{O}) + 3\text{H}_2\text{O} \quad K_{c2} \quad (13)$$

For octopamine:

$$\text{H}_2\text{L}(\text{H}_2\text{O})_3 + \text{OH}(\text{H}_2\text{O})_3 \text{L}^- \rightleftharpoons (\text{H}_2\text{O})_4 + 3\text{H}_2\text{O} \quad K_{d2} \quad (14)$$

In the above reaction, $\text{L}(\text{H}_2\text{O})$ and $\text{HL}(\text{H}_2\text{O})_4$ represent the 6-APA and octopamine anion solvated with one and four water molecule respectively. The reaction described in Eqs 13 and 14 are characterized by the equilibrium constants $K_{c2}$ and $K_{d2}$, which were also theoretically determined. Combining Eqs 4 and 13 or Eqs 8 and 14, the second ionization reaction of 6-APA and octopamine were obtained.

For 6-APA:

$$\text{HL}(\text{H}_2\text{O})_3:Z \rightleftharpoons \text{L}(\text{H}_2\text{O}) + \text{H}_2\text{O} + \text{H}_3\text{O}^+ \quad K_{a2} \quad (15)$$

For octopamine:

$$\text{H}_2\text{L}(\text{H}_2\text{O})_3 + 2\text{H}_2\text{O} \rightleftharpoons \text{HL}(\text{H}_2\text{O})_4 + \text{H}_3\text{O}^+ \quad K_{b2} \quad (16)$$

The equilibrium constants, $K_{a2}$ and $K_{b2}$, that characterize the above reaction are given by Eqs 17 and 18.

$$K_{a2} = K_{c2} \times K_N \quad (17)$$

$$K_{b2} = K_{d2} \times K_M \quad (18)$$

These equations were used to obtain the value of the second ionization constant of 6-APA and
it is observed that $N_{11}$ of neutral electronic density decreases with respect to $N_{11}$ atom of octopamine anion (Tables 3 and 4).

Also, the theoretically calculated $pK_a$ values ($pK_a = 4.77$ and $4.90$ for 6-APA and octopamine respectively) are comparable with the experimentally determined values of $pK_a$ ($pK_a = 9.80$ and $9.53$) [29,31].

**Third ionization constant of octopamine**

There is a third ionization constant for octopamine and this $pK_a$ value is due to the anion $HL^-$ undergoing total neutralization as follows:

$$HL^- + OH^-(H_2O)_4 \rightleftharpoons L^2-(H_2O)_4 + K_{d3}$$

In the above reaction, $L^2-(H_2O)_4$ represents the octopamine anion solvated with four water molecules. By combining Eqs 19 and 8, we obtain the reaction given in Eq. 20, which defines the third ionization constant of octopamine ($K_{d3}$) and shows the solvation of the anion octopamine.

$$HL^- + 5(H_2O) \rightleftharpoons (H_2O)_4 + H_2O^+ + K_{d3}$$

It is evident that the constants $K_{d3}$, $K_M$, and $K_{b1}$ are related as shown in Eq. (21).

$$K_{b3} = K_{d3} \times K_M$$

The above equation was used to determine the theoretical value of the third ionization constant of octopamine in water. Table 1 summarizes the optimized values of molecular properties of the $HL^-$ anion, $OH^- (H_2O)_3$ ion, and $L^2-(H_2O)_4$ anion molecule obtained at the B3LYP/6-31+G(d) level of theory with Tomasi’s method in water at 298.15 K.

Also, it was observed that for octopamine $O_9$ of anion ($HL^-$), electronic density increases with respect to $O_9$ atom of octopamine anion ($L^2-(H_2O)_4$) (Tables 3 and 4).

The water molecule originating from the acid-base reaction, together with the hydration water molecule of the 6-APA and octopamine interact with the 6-APA and octopamine molecules by intermolecular hydrogen bonds. The distances and angles that characterize these intermolecular hydrogen bonds (Tables 3 and 4) indicate that they belong to the class of weak, closely to moderate and moderate intermolecular hydrogen bond. According to earlier studies [26,30], the moderate hydrogen bonds have the following characterization: bond lengths of $H \cdots B$ are between 1.5 and 2.2 Å, and the bond angle is

| Table 3 – Calculated structural magnitudes using Tomasi’s method at the B3LYP/6-31+G(d) level of theory for the cation, neutral molecule and anion of 6-APA at 298.15 K |
|---|---|---|
| **6-APA** | **Calc. magnitudes** | **H$_2$L$^-$($H_2$O)$_4$** | **HL$^-$($H_2$O)$_4$** | **L$^-$($H_2$O)$_4$** |
| d O$_{26}$-H$_{37}$ | 1.67414 | - | - |
| d O$_{15}$-H$_{37}$ | 1.00567 | - | - |
| d O$_{15}$-C$_{13}$ | 1.33231 | - | - |
| d O$_{26}$-C$_{13}$ | 1.21421 | - | - |
| d O$_{26}$-H$_{38}$ | - | 1.97884 | - |
| d O$_{15}$-H$_{32}$ | - | 1.99017 | - |
| d O$_{15}$-H$_{33}$ | - | 1.77715 | - |
| d O$_{15}$-H$_{29}$ | - | 1.79861 | - |
| d O$_{15}$-C$_{13}$ | - | - | 1.25893 |
| d O$_{15}$-H$_{28}$ | - | - | 2.04792 |
| d O$_{15}$-H$_{27}$ | - | - | 2.03860 |
| d O$_{15}$-C$_{13}$ | - | - | 1.25966 |
| A H$_{29}$-O$_{26}$-H$_{37}$ | 129.20485 | - | - |
| A H$_{29}$-O$_{26}$-H$_{37}$ | 104.48587 | - | - |
| A O$_{26}$-H$_{37}$-O$_{15}$ | 165.38296 | - | - |
| A O$_{15}$-C$_{13}$-O$_{14}$ | 125.50181 | - | - |
| A H$_{37}$-O$_{15}$-C$_{13}$ | 108.62947 | - | - |
| A H$_{37}$-O$_{15}$-C$_{13}$ | - | 111.23369 | - |
| A O$_{15}$-H$_{31}$-O$_{14}$ | - | 164.30729 | - |
| A O$_{15}$-H$_{32}$-O$_{15}$ | - | 158.12370 | - |
| A O$_{15}$-H$_{32}$-O$_{14}$ | - | 171.32960 | - |
| A H$_{37}$-O$_{14}$-C$_{13}$ | - | 116.86130 | - |
| A H$_{37}$-O$_{14}$-C$_{13}$ | - | 117.67696 | - |
| A O$_{26}$-H$_{37}$-O$_{14}$ | - | - | 142.86930 |
| A O$_{15}$-H$_{32}$-O$_{16}$ | - | - | 142.18373 |
| A O$_{15}$-C$_{13}$-O$_{14}$ | - | - | 129.04127 |
| d O$_{15}$ | -0.981 | - | - |
| d O$_{16}$ | -0.687 | -0.561 | -0.638 |
| d O$_{14}$ | -0.437 | -0.616 | -0.613 |
| d C$_{13}$ | 0.519 | 0.302 | 0.594 |
| d O$_{11}$ | - | -1.039 | - |
| d O$_{14}$ | - | -1.058 | - |
| d O$_{27}$ | - | -1.111 | - |
| d O$_{26}$ | - | - | -1.005 |
| d N$_{9}$ | -0.955 | -0.932 | -0.760 |
130°–180°. For weak hydrogen bonds, the bond length and angle are 2.2–3.2 Å and 90°–150°, respectively, and for strong hydrogen bonds are 1.2–1.5 Å and 175°–180°, respectively. The intermolecular hydrogen bond of the all species of octopamine drug molecule, the three nucleophilic attack on the hydrogen atoms of NH$_3^+$ groups was observed, while for 6-APA drug molecules, the three nucleophilic attack on the hydrogen atoms of NH$_3^+$ and two OH groups was observed. The calculations performed at the B3LYP/6-31+G(d) levels of theory using Tomasi’s method prove that cations, neutral molecules, and anion of octopamine at 298.15 K.

### Conclusions

The feasibility of a theoretical method that uses $pK_a$ values to determine the ionization constants of octopamine and 6-APA is discussed. It is shown that these constants can be calculated with an acceptable degree of accuracy. Various acid-base reactions were considered, which take into account the solvation of the hydrogen, hydroxyl ions, and other cations or anions in protic solvents such as water, which possess a high hydrogen-bond-donor capability. For 6-APA drug molecules, the nucleophilic attack on the hydrogen atoms of the COOH and NH$_3^+$ groups was observed, while for octopamine drug molecule, the three nucleophilic attack on the hydrogen atoms of NH$_3^+$ and two OH groups was observed. The calculations performed at the B3LYP/6-31+G(d) levels of theory using Tomasi’s method prove that cations, neutral molecules, and anions form intermolecular hydrogen bonds with some molecules of water. It is shown that theoretically calculated $pK_a$ values are in good agreement with the existing experimental $pK_a$ values, which are determined from potentiometric titration and UV-visible spectrophotometric measurements.

### References

24. Gaussian 09, Revision A.1, (Gaussian, Inc., Wallingford, CT) 2009.