

**DETERMINATION OF ACIDIC DISSOCIATION CONSTANTS OF ASPARTIC ACID AND GLYCYL ASPARTIC ACID IN WATER USING AB INITIO AND DFT METHODS**

**Farhoush Kiani<sup>a,\*</sup> and Belgheys Vahidi<sup>a</sup>**

<sup>a</sup> Department of Chemistry, Faculty of Science, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran

\*Corresponding author e-mail address: farhoush\_kiani@yahoo.com

**ABSTRACT**

The pK<sub>a</sub> values for two biomolecules, aspartic acid (Asp) and glycylasspartic (GlyAsp) were calculated using Ab initio method in aqueous solution at ambient temperature. To explain the acidic dissociation constants determined, we investigated the molecular conformations and solute-solvent interactions of the species of aspartic acid and glycylasspartic acid were investigated using density functional theory (DFT) method. The basis set at the B3LYP/6-31+G(d) level of theory were selected for calculations of peptides and amino acid, by the density functional theory (DFT). We selected the basis set at the B3LYP/6-31+G(d) level of theory for calculations. Tomasi's process was used to analyze the formation of intermolecular hydrogen bonds between cationic, anionic and neutral species of amino acid, peptide and water molecules. Several ionization reactions and equilibria in aqueous possessing a high hydrogen-bond-donor capability, were considered and the results showed that in alkaline aqueous solution the cation, anion, and neutral species of aspartic acid and glycylasspartic acid are solvated with one, two, three, and four molecules of water, respectively. Atomic charges were applied to study the reaction mechanism. The pK<sub>a</sub> values calculated theoretically, showed a good agreement, with the experimentally reported pK<sub>a</sub> values in the literature.

**KEYWORDS:** Ab initio, Dissociation Constant, DFT, Aspartic acid, Glycyl aspartic Acid.

**INTRODUCTION**

Peptides are increasingly attractive as therapeutics gives their diverse biological functions and potentially high potency and target specificity (Chereau and Allary, 2005). In the near future, an increasing activity in design and synthesis of new peptide-based drugs is expected, as a result of combined advances in proteomic research and biotechnology (Catsch and Harmuth-Hoene, 1976). Peptide structures that have been determined have come from analysis of NMR or other experimental data in many cases supplemented with theoretical modeling. Amino acids can form not only variable peptide chains but also three-dimensional crystalline structures, which are no less variable and beautiful than biopolymers. Amino acids belong to systems that are replete with mystery and magic and that serve as beautiful models. These small molecules form the elementary basis of peptides and proteins, which are crucial for life. This condition alone would suffice to make the study of amino acids exciting and important, but studies of amino acids are not restricted to biological problems (Beachy *et al.*, 1976).

Aspartic acid CH(NH<sub>2</sub>)(COOH)CH<sub>2</sub>(COOH) is one of the naturally occurring amino acids, with an additional carboxylic group in the side chain (Aue *et al.*, 1979; Bregier-Jarzebowska *et al.*, 2001). Similarly as glutamine acid, it belongs to the main neurotransmitters in the central nervous system. Aspartic acid takes part in the thermogenic processes induced by prostaglandin (Monda *et al.*, 1998) and is a component of the active centers of some enzymes. D-Aspartic acid (DA), an acidic amino acid, is synthesized in the body from either endogenous microflora or by spontaneous racemization of the L-isomeric form (Timme and Collins, 2002). Free D-aspartate is present in a wide variety of cells and tissues, particularly the central nervous system and endocrine organs. During early development, concentrations of DA increase transiently, but gradually decrease to trace amounts in adults (Fuchs *et al.*, 2005; Kera *et al.*, 1995). Suggesting a morphological and functional role of DA during maturation of these organs (Hashimoto *et al.*, 1995; Dunlop *et al.*, 1986) D-Aspartic acid is synthesized within the seminiferous tubules, secreted into the venous blood, and preferentially localized in Leydig cells, the site of testosterone synthesis (D'Aniello *et al.*, 1998; Sakai *et al.*, 1998). Besides its steroidogenic role, DA has been shown to increase the secretion of prolactin and LH (D'Aniello *et al.*, 1998; D'Aniello *et al.*, 1996).

These studies proton transfer reactions constitute an important class of chemical reactions and have been studied extensively over a long period of time (Aue *et al.*, 1979). In a gas phase and solution phase provides information on inherent substituent effects and solute-solvent interactions. The study of acidities in the gas phase allows assessment of



## RESULTS AND DISCUSSION

Amino acids are small organic molecules that have both carboxylic amino groups (e. g. of general formula  $\text{NH}_2 - \text{CH}(\text{R}) - \text{COOH}$ ). Their side chain R, can vary small or bulky, hydrophobic or hydrophilic, polar, charged or neutral. In both aqueous solution and the crystalline state amino acids exist as zwitterions, in which both the amino and carboxylic groups ionized ( $\text{NH}_3^+ - \text{CH}(\text{R}) - \text{COO}^-$ ). In matrices of noble gas they can be obtained in their non-ionized form (Boeyens and Ogilive, 2008). The solubility of amino acids in water is highly variable. Addition of acids or bases improves the solubility through salt formation. The dissociation constants of amino acids can be determined, for example, by titration of the acid. In amino acids the acidity of the carboxyl group is higher and the basicity of the amino group lower than in the corresponding carboxylic acids and amines. The acidity of the free carboxyl groups and the basicity of the free amino groups are lower in peptides than in the corresponding free amino acids (Belitz *et al.*, 2009). The aspartic acid and glycyaspartic acid have tendency to lose three acidic protons. For aspartic acid, first 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, this concept of microscopic ionization constants  $K_1$  and  $K_2$  may be applied, where  $K_1$  involving the carboxyl proton is (Laitinen and Harris, 1975):

$$K_1 = \frac{[H^+][\text{NH}_2\text{CHR}\text{COO}^-]}{[\text{NH}_3^+\text{CHR}\text{COOH}]} \quad (1)$$

and  $K_2$  involving the ammonium proton is:

$$K_2 = \frac{[H^+][\text{NH}_3^+\text{CHR}\text{COOH}]}{[\text{NH}_3^+\text{CHR}\text{COOH}]} \quad (2)$$

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  $(k_{12} \cdot k_{21}) / (k_{12}+k_{21})$ , where the subscript 21 notes loss of proton 2 following loss of proton 1 and subscript 12 denotes loss of proton 1 following by 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 and second K correspond to removal of the carbonyl proton and from the ammonium almost, but not completely, exclusively. It can be determined by NMR spectroscopy exactly (Laitinen and Harris, 1975; Rabenstein and Sayer, 1976).

Considering eqs 1 and 2, different reactions including cationic, neutral, and anionic species were tested (see Table 1), 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 constants of Asp & GlyAsp have been determined using the potentiometric technique. The method of determining acidic dissociation constants was previously described, and its values were used in this work. These values are listed in Table 2 together with the calculated values using the Tomasi method at the B3LYP/6-31+G(d) level of theory.

**Table 1. Calculated total energy using the Tomasi method at the B3LYP/6-31+G(d) level of theory for cationic, neutral, and anionic species of aspartic acid and glycyaspartic acid at 298.15K<sup>a</sup>.**

No.	Solvated Species (Aspartic acid)	$G_{\text{sol}}^0$ (Hartree)	$G_{\text{sol}}^0$ /molecule (Kj.mol-1)	Solvated Specie (Glycyaspartic acid)	$G_{\text{sol}}^0$ (Hartree)	$G_{\text{sol}}^0$ /molecule (Kj.mol-1)
0	$\text{H}_3\text{L}^+$	-512.810170	-1593736.221	$\text{H}_3\text{L}^+$	-720.828704	-1047566.41
1	$\text{H}_3\text{L}^+(\text{H}_2\text{O})$	-589.255503	-897209.7171	$\text{H}_3\text{L}^+(\text{H}_2\text{O})$	-797.267554	-624125.9105
2	$\text{H}_3\text{L}^+(\text{H}_2\text{O})_2$	-665.700586	-665044.1219	$\text{H}_3\text{L}^+(\text{H}_2\text{O})_2$	-873.736099	-482976.6837
3	$\text{H}_3\text{L}^+(\text{H}_2\text{O})_3$	-742.126749	-548968.1492	$\text{H}_3\text{L}^+(\text{H}_2\text{O})_3$	-950.176833	-412404.661
4	$\text{H}_3\text{L}^+(\text{H}_2\text{O})_4$	-818.588113	-479312.0757	$\text{H}_3\text{L}^+(\text{H}_2\text{O})_4$	-1026.618350	-370067.1048

**Table 1: Continued**

0	H <sub>2</sub> L	-512.364077	-1593738.067	H <sub>2</sub> L	-720.378486	-1047570.396
1	H <sub>2</sub> L (H <sub>2</sub> O)	-588.811649	-897202.388	H <sub>2</sub> L (H <sub>2</sub> O)	-796.830523	-624145.6516
2	H <sub>2</sub> L (H <sub>2</sub> O) <sub>2</sub>	-665.232671	-665051.1206	H <sub>2</sub> L (H <sub>2</sub> O) <sub>2</sub>	-873.263650	-482992.7211
3	H <sub>2</sub> L (H <sub>2</sub> O) <sub>3</sub>	-741.681657	-548968.1492	H <sub>2</sub> L (H <sub>2</sub> O) <sub>3</sub>	-949.718215	-412418.6608
4	H <sub>2</sub> L (H <sub>2</sub> O) <sub>4</sub>	-818.142044	-479313.9881	H <sub>2</sub> L (H <sub>2</sub> O) <sub>4</sub>	-1026.180777	-370071.8633
0	HL <sup>-</sup>	-511.917050	-1594930.661	HL <sup>-</sup>	-719.938128	-1048753.441
1	HL <sup>-</sup> (H <sub>2</sub> O)	-588.360857	-897805.9996	HL <sup>-</sup> (H <sub>2</sub> O)	-796.378248	-624725.635
2	HL <sup>-</sup> (H <sub>2</sub> O) <sub>2</sub>	-664.786935	-665445.202	HL <sup>-</sup> (H <sub>2</sub> O) <sub>2</sub>	-872.829209	-483381.1341
3	HL <sup>-</sup> (H <sub>2</sub> O) <sub>3</sub>	-741.232091	-549264.1881	HL <sup>-</sup> (H <sub>2</sub> O) <sub>3</sub>	-949.273463	-412705.0543
4	HL <sup>-</sup> (H <sub>2</sub> O) <sub>4</sub>	-817.702854	-479549.3663	HL <sup>-</sup> (H <sub>2</sub> O) <sub>4</sub>	-872.829209	-370310.6998
0	L <sup>2-</sup>	-511.434750	-1592581.841	L <sup>2-</sup>	-719.460065	-1046390.239
1	L <sup>2-</sup> (H <sub>2</sub> O)	-587.896873	-896629.6011	L <sup>2-</sup> (H <sub>2</sub> O)	-795.899746	-623539.7309
2	L <sup>2-</sup> (H <sub>2</sub> O) <sub>2</sub>	-664.318334	-664661.5752	L <sup>2-</sup> (H <sub>2</sub> O) <sub>2</sub>	-872.375326	-482589.4155
3	L <sup>2-</sup> (H <sub>2</sub> O) <sub>3</sub>	-740.749248	-548670.94	L <sup>2-</sup> (H <sub>2</sub> O) <sub>3</sub>	-948.818362	-412111.0304
4	L <sup>2-</sup> (H <sub>2</sub> O) <sub>4</sub>	-817.232380	-479077.9342	L <sup>2-</sup> (H <sub>2</sub> O) <sub>4</sub>	-1025.265961	-369837.0759

<sup>a</sup> *N*: total number of solvation water molecules;  $G_{sol}^0$ , total free energy in solution;  $G_{sol}^0$ /molecule, total energy of solvated species per water molecule; H<sub>2</sub>L<sup>+</sup>, cation species; HL<sup>-</sup>, neutral; L<sup>-</sup>, anion species.

### Solvent- Solute Interactions

**Ionic product of water.** It is well-known that all aqueous solutions contain hydrogen (H<sup>+</sup>) and hydroxyl (OH<sup>-</sup>) ions. In pure water these ions are derived completely from the ionization of the water molecules:



Considering that the H<sup>+</sup> ion is hydrated, appearing predominantly as H<sub>3</sub>O<sup>+</sup>, the autoprotolysis of water is better represented by the reaction:



Taking into account that water is only slightly dissociated and to simplify the discussion, we shall make the approximations of replacing the activities in acidity constants by the numerical values of the molar concentrations. Consequently:

$$K_w = [H_3O^+][OH^-] \quad (5)$$

At 298.15 K,  $K_w = 1.008 \cdot 10^{-14}$ , showing that only a few of the water molecules are ionized (Atkins, 1998).

Conventionally, eqs 4 and 5 are those more used in studies of acid-base equilibria in aqueous media. On the other hand, the solvation of anions is effective in protic solvents where hydrogen bonds may be formed between the proton of the solvent and the lone pairs of electrons of the anion (Jeffrey, 1997). The total energies of the single and solvated OH<sup>-</sup> ion have been calculated in water at the B3LYP/6-31+G(d) level of theory, using Tomasi's model.

Considering these facts and to provide a more satisfactory representation of the protolysis of water, the reaction has been shown as follows:



The selected reaction considers that both H<sup>+</sup> and OH<sup>-</sup> ions are hydrated with one water molecule. Moreover, indicating with  $K_N$  the equilibrium constant of the reaction of eq. 6 and taking into account eqs 4 and 5, it is inferred that (Blanco *et al.*, 2005):

$$K_w = K_N [H_2O]$$

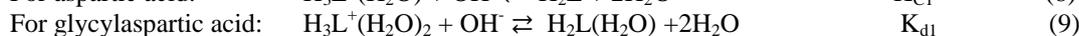
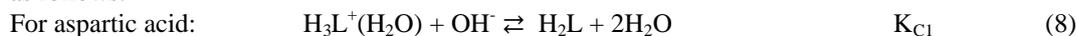
Where [H<sub>2</sub>O] is the molar concentration of water. Consequently, at 298.15 K, it was calculated that:

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

The total energies of the single and solvated aspartic acid 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 variations of the total energy (kJ·mol<sup>-1</sup>) of the species per water molecule as a function of the total number of solvation water molecules. Figure 2 and Table 1 show the marked decreases of the total energies of ions when the solvation increases.

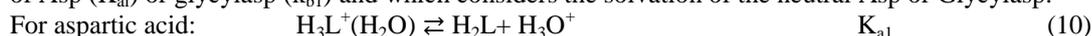
**First ionization constant of aspartic acid and glycyaspartic acid:**

It was selected that in alkaline solutions Aspartic acid and glycyaspartic acid suffers a reaction of partial neutralization as follows:



In this reaction,  $H_3L^+(H_2O)$  is the Asp cation solvated with one water molecule,  $H_2L$  represents Asp natural solvated,  $H_3L^+(H_2O)_2$  and  $H_2L(H_2O)$  are glycyasp cation solvated two and neutral solvated one water molecules, respectively. The previous reactions are characterized by equilibrium constants,  $K_{C1}$  and  $K_{d1}$ , which were theoretically determined.

By combining eqs. 8 and 4 or 4 and 9, we obtain the reaction of eq 10 or 11 which defines the first ionization constant of Asp ( $K_{a1}$ ) or glycyasp ( $k_{b1}$ ) and which considers the solvation of the neutral Asp or Glycyasp.



It is evident that:

$K_{a1} = K_{c1} \times K_w$

Or

$K_{b1} = K_{d1} \times K_w$

The above equations were used to determine theoretically the value of the first ionization constant in water. Table 2 summarizes the optimized values of molecular properties of the  $H_3L^+(H_2O)$  cation,  $H_2L$  neutral molecule for Asp (Figure 2).

And  $H_3L^+(H_2O)_2$  cation,  $H_2L(H_2O)$  neutral for glycyasp (Figure 4) obtained at the B3LYP/6-31+ G(d) level of theory with Tomasi's method in water at 298.15K. It must be noted that in the formation of the neutral aspartic acid solvated without water molecules the neutral molecules practically do not have the structure that characterizes the solvated Aspartic acid cation (Figure 2 and Table 3).

Obviously, the formation of the Asp cation implies that the electronic density of the O9 atom increase notably (in absolute value) with respect to the O<sub>9</sub> atom of the neutral Asp, and also for glycyasp observe that O11 of cation electronic density decrease to do resonance respect to O11 atom of glycyasp neutral (Table 3 and 4).

The  $pK_{a1}$  value (Asp and glycyasp) theoretically obtained ( $pK_{a1}=2.58$  and 2.55) is relatively comparable with the experimental  $pK_{a1}$  value ( $pK_{a1}=2.09$  and 2.88) (Dean, 1999).

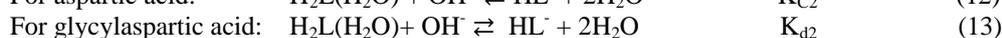
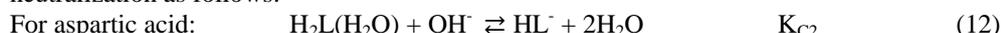
**Table 2. Values of  $pK_a$  for the protonation of aspartic acid and glycyaspartic acid obtained using the Tomasi method at the B3LYP/6-31+G(d) level of theory, at 298.15 K<sup>a</sup>.**

species	selected equations	$pK_a$ (calculated) this work	$pK_a$ (experimental)	ref
Aspartic acid	$H_3L^+(H_2O) \rightleftharpoons H_2L + H_3O^+$	2.584324236	1.89 (I=0)	39
	$H_2L(H_2O) \rightleftharpoons HL^- + H_3O^+$	3.180192634	3.65 (I=0)	39
	$HL^-(H_2O) + H_2O \rightleftharpoons L^{2-}(H_2O) + H_3O^+$	9.650936629	9.60 (I=0)	39
Glycyaspartic acid	$H_3L^+(H_2O)_2 \rightleftharpoons H_2L(H_2O) + H_3O^+$	2.553932185	2.81 (I=0)	39
	$H_2L(H_2O) \rightleftharpoons HL^- + H_3O^+$	4.794194748	4.45 (I=0)	39
	$HL^-(H_2O) \rightleftharpoons L^{2-} + H_3O^+$	8.393486916	8.60 (I=0)	39

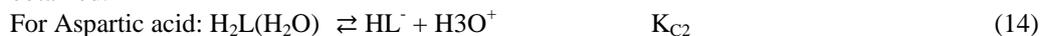
<sup>a</sup> I, ionic strength.

**Second ionization constant of aspartic acid and glycyaspartic acid:**

Here, there is selected that the neutral  $H_2L(H_2O)$  (for aspartic acid) and  $H_2L(H_2O)$  (for glycyaspartic acid) suffers total neutralization as follows:



In the above reaction,  $HL^-$  represents the Asp and glycyasp anion solvated without water molecule. The reaction described in eq 12 and 13 are characterized by another equilibrium constant,  $K_{C2}$  and  $K_{d2}$ , which was also theoretically determined. Combining eqs 4 and 12 or eqs 4 and 13, the second ionization reaction of aspartic acid and glycyasp were obtained.



Equilibrium constant  $K_{a2}$  and  $K_{b2}$  that characterizes the above reaction are:

$$K_{a2} = K_{C2} \times K_w \quad (16)$$

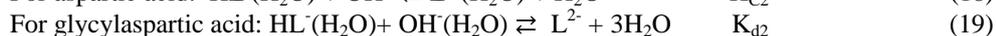
$$K_{b2} = K_{d2} \times K_w \quad (17)$$

These equations were used to obtain the value of the second ionization constant of Aspartic acid and glycyaspatic acid in water. Table 3 and 4 gives the values of the molecular parameters and properties calculated for the  $HL^-$  anion and  $H_2L(H_2O)$  neutral, in water at 298.15 K, while Figures 2 and 3 shows the structures of these anion and cation. The ASP and GlyASP anion solvated with water molecule possesses various structural characteristics that are different from those of the neutral ASP and GlyASP molecule and the ASP and GlyASP cation solvated with water molecules, respectively. Obviously, the formation of the neutral Asp implies that the electronic density of the O7 atom decrease to do resonance notably (in absolute value) with respect to the O7 atom of the Asp neutral, and also for glycyasp observe that O13 of anion electronic density increase respect to O13 atom of glycyasp anion (Table 3 and 4).

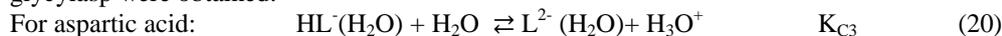
Also the  $pK_{a2}$  value (Asp and glycyasp) theoretically calculated ( $pK_{a2}=3.18$  and  $4.79$ ) for is relatively comparable with the experimentally determined  $pK_{a2}$  ( $pK_{a2}=3.86$  and  $4.45$ ) (Dean, 1999).

#### Third ionization constant of aspartic acid and glycyaspatic acid :

Here, there is third that the anion  $HL^-(H_2O)$  (for aspartic acid) and  $HL^-(H_2O)$  (for glycyaspatic acid) suffers total neutralization as follows:



In the above reaction,  $L^{2-}(H_2O)$  and  $L^{2-}$  represents the Asp and glycyasp anion solvated with one and without water molecule. The reaction described in eq 18 and 19 are characterized by another equilibrium constant,  $K_{C3}$  and  $K_{d3}$ , which was also theoretically determined. Combining eqs 4 and 18 or eqs 6 and 19, the Third ionization reaction of Asp and glycyasp were obtained.



The equilibrium constant  $K_{a3}$  and  $K_{b3}$  that characterizes the above reaction, are:

$$K_{a3} = K_{C3} \times K_w \quad (22)$$

$$K_{b3} = K_{d3} \times K_N \quad (23)$$

These equations were used to obtain the value of the second ionization constant of Aspartic acid and glycyaspatic acid in water. Table 3 and 4 gives the values of the molecular parameters and properties calculated for the  $HL^-(H_2O)$ ,  $L^{2-}(H_2O)$  and  $L^{2-}$  anions, in water at 298.15 K, while Figures 2 and 3 shows the structures of these anions. The Asp and GlyASP anion solvated with water molecule possesses various structural characteristics that are different from those of the neutral Asp and GlyASP molecule and the Asp and GlyASP cation solvated with water molecules, respectively.

Obviously, the formation of the anion  $HL^-(H_2O)$  Asp implies that the electronic density of the N5 atom decrease notably (in absolute value) with respect to the N5 atom of the Asp  $L^{2-}(H_2O)$  anion, and also for glycyasp observe that N8 of neutral electronic density decrease respect to N8 atom of glycyaspatic acid anion (Table 3 and 4).

Also the  $pK_{a3}$  value (aspartic acid and glycyaspatic acid) theoretically calculated ( $pK_{a3}=3.18$  and  $4.79$ ) for is relatively comparable with the experimentally determined  $pK_{a3}$  ( $pK_{a3}=3.86$  and  $4.45$ ) (Dean, 1999).

The molecule of water originated from the acid-base reaction, together with the hydration water molecule of the aspartic acid and glycyasp, and these are the molecules of water that interact with the Asp and glycyasp molecules by means of IHBs. The distances and angles that characterize these IHBs (Table 3 and 4) indicate that they belong to the class of weak to moderate and moderate IHB. According to references (Jeffrey, 1997; Kiani *et al*, Article in Press) the properties of the moderate hydrogen bonds have the following characterization: bond lengths of  $H \cdots B$  are between (1.5



**Table 3.** Calculated structural magnitudes using Tomasi's method at the B3LYP/6-31+G(d) level of theory for the cation, zwitterion molecule, and anion of aspartic acid at 298.15 K<sup>a</sup>.

Aspartic acid	H <sub>3</sub> L <sup>+</sup> (H <sub>2</sub> O)	H <sub>2</sub> L(H <sub>2</sub> O)	L <sup>2-</sup> (H <sub>2</sub> O)	HL <sup>-</sup> (H <sub>2</sub> O)	H <sub>2</sub> L	HL <sup>-</sup>
K <sub>a1</sub>	0.002604209	-	-	-	-	-
K <sub>a2</sub>	0.0006604	-	-	-	-	-
K <sub>a3</sub>	2.2339*10 <sup>-10</sup>	-	-	-	-	-
K <sub>c1</sub>	2.58354*10 <sup>11</sup>	-	-	-	-	-
K <sub>c2</sub>	6551591848	-	-	-	-	-
K <sub>c3</sub>	22161.6881	-	-	-	-	-
a <sub>0</sub>	4.39	4.54	4.45	4.50	4.03	4.07
D-C <sub>4</sub> C <sub>3</sub> C <sub>2</sub> C <sub>1</sub>	173.443075	173.610806	177.380225	178.160710	168.067865	-176.613097
D-N <sub>5</sub> C <sub>3</sub> C <sub>2</sub> C <sub>1</sub>	-66.929785	-67.240373	-59.073659	-55.379947	-66.348143	-54.342958
D-O <sub>6</sub> C <sub>1</sub> C <sub>2</sub> C <sub>3</sub>	-152.391337	-132.289959	47.935510	-147.214007	-4.590266	-142.629299
D-O <sub>7</sub> C <sub>1</sub> O <sub>6</sub> C <sub>2</sub>	178.103033	178.695773	-177.750203	177.925689	179.788025	177.762681
D-O <sub>8</sub> C <sub>4</sub> C <sub>3</sub> C <sub>2</sub>	117.770321	116.870657	89.713128	118.426726	148.309697	102.210823
D-O <sub>9</sub> C <sub>4</sub> O <sub>3</sub> C <sub>2</sub>	-62.627743	-62.442546	-88.934208	-60.925396	-33.819379	-76.316050
DH <sub>10</sub> C <sub>2</sub> C <sub>1</sub> C <sub>6</sub>	-30.981187	103.751946	-	-23.403924	117.228111	-18.699556
DH <sub>11</sub> C <sub>2</sub> C <sub>1</sub> C <sub>6</sub>	83.506817	52.254592	-	91.348244	-128.246995	96.515666
DH <sub>12</sub> C <sub>3</sub> C <sub>2</sub> C <sub>1</sub>	51.996731	-81.685129	61.426674	60.991948	-53.120915	64.456854
DH <sub>13</sub> N <sub>5</sub> C <sub>3</sub> C <sub>2</sub>	177.220495	41.953673	-83.853924	165.9839929	79.570844	153.266260
qN <sub>5</sub>	-1.189938	-1.068521	-0.992093	-1.105901	-0.989915	-1.014638
qO <sub>6</sub>	-0.546105	-0.543478	-0.752980	-0.617335	-0.573992	-0.617835
qO <sub>7</sub>	-0.539702	-0.708072	-0.763330	-0.646902	-0.652127	-0.646698
qO <sub>8</sub>	-0.739492	-0.757015	-0.769389	-0.785060	-0.623871	-0.734808
qO <sub>9</sub>	-0.503409	-0.669033	-0.0750695	-0.706516	-0.587156	-0.725228
qO <sub>18</sub>	-1.069031	-	-	-	-	-
qH <sub>10</sub>	0.296053	0.282301	0.219853	0.260234	0.283882	0.256444
qH <sub>11</sub>	0.313207	0.294694	0.235986	0.274678	0.281115	0.273113
qH <sub>14</sub>	0.620100	0.539467	0.432366	0.246054	0.546773	0.438610
qH <sub>15</sub>	0.556566	0.563548	-1.083088	0.560830	0.451497	0.560332
qH <sub>16</sub>	0.603190	0.584469	0.533762	-1.112600	-0.551080	-
qH <sub>17</sub>	0.569470	-1.105872	0.537111	0.582147	-	-
d-C <sub>2</sub> C <sub>1</sub>	1.523084	1.514005	1.563576	1.531484	1.511368	1.529428
d-C <sub>3</sub> C <sub>2</sub>	1.540185	1.533394	1.556806	1.543481	1.532195	1.545097
d-C <sub>4</sub> C <sub>3</sub>	1.532261	1.584989	1.5566273	1.569466	1.545088	1.574073
d-N <sub>5</sub> C <sub>3</sub>	1.506367	1.512043	1.476687	1.484436	1.470405	1.490438
d-O <sub>6</sub> C <sub>1</sub>	1.199101	1.205839	1.271887	1.220132	1.215662	1.220386
d-O <sub>7</sub> C <sub>1</sub>	1.373472	1.378407	1.264484	1.341554	1.352938	1.433224
d-O <sub>8</sub> C <sub>4</sub>	1.212041	1.271648	1.272085	1.274037	1.341556	1.264226
d-O <sub>9</sub> C <sub>4</sub>	1.331045	1.231820	1.269436	1.246716	1.210458	1.252457
d-H <sub>10</sub> C <sub>2</sub>	1.092769	1.091713	1.096026	1.090954	1.096452	1.091688
d-H <sub>11</sub> C <sub>2</sub>	1.098775	1.100294	1.102277	1.102041	1.098244	1.101395
d-H <sub>12</sub> C <sub>3</sub>	1.09497	1.093095	1.097959	1.097448	1.097339	1.0966026
d-H <sub>13</sub> N <sub>5</sub>	1.025428	1.032389	1.021748	1.000000	1.000000	1.000000
d-H <sub>14</sub> N <sub>5</sub>	1.053873	1.025569	1.025617	1.000000	1.000000	1.000000
d-H <sub>15</sub> N <sub>5</sub>	1.030525	1.041896	-	-	-	1.000000
d-H <sub>16</sub> N <sub>7</sub>	0.978805	-	-	-	-	-

**Table 3: Continued**

d-H <sub>17</sub> O <sub>9</sub>	0.978888	-	-	-	-	-
d-H <sub>18</sub> N <sub>5</sub>	2.760089	-	-	-	-	-
d-H <sub>19</sub> O <sub>18</sub>	0.972440	-	-	-	-	-
d-H <sub>20</sub> O <sub>18</sub>	0.971417	-	-	-	-	-
d-N <sub>5</sub> H <sub>19</sub>	3.24489	2.67479	-	-	-	-
d-O <sub>8</sub> H <sub>18</sub>	-	3.33790	-	-	-	-
d-N <sub>5</sub> H <sub>18</sub>	-	3.58641	-	-	-	-
d-O <sub>8</sub> H <sub>17</sub>	-	-	1.95983	3.08129	-	-
d-O <sub>18</sub> H <sub>15</sub>	3.30927	-	-	-	-	-
d-O <sub>9</sub> H <sub>16</sub>	-	-	1.93625	-	-	-
d-N <sub>5</sub> H <sub>17</sub>	-	-	-	3.91966	-	-
d-O <sub>17</sub> H <sub>15</sub>	-	1.82317	-	-	-	-
A-C <sub>3</sub> C <sub>2</sub> C <sub>1</sub>	116.103888	116.401620	116.674064	116.046112	112.274144	116.140677
A-C <sub>4</sub> C <sub>3</sub> C <sub>2</sub>	112.356571	110.527724	109.382415	110.201475	110.781314	109.394719
A-N <sub>5</sub> C <sub>3</sub> C <sub>2</sub>	111.370224	111.854774	113.679414	109.101301	116.179996	109.349716
A-O <sub>6</sub> C <sub>1</sub> C <sub>2</sub>	124.641609	126.631505	116.998822	122.660330	125.754449	123.191201
A-O <sub>7</sub> C <sub>1</sub> O <sub>6</sub>	123.114510	121.337613	126.426404	121.311807	122.494705	121.218648
A-O <sub>8</sub> C <sub>4</sub> C <sub>3</sub>	122.795259	113.583609	116.954747	115.936874	113.704785	114.522540
A-O <sub>9</sub> C <sub>4</sub> C <sub>3</sub>	111.018480	114.665899	117.252630	115.637838	123.286895	115.526432
A-H <sub>10</sub> C <sub>2</sub> C <sub>1</sub>	106.949135	108.031253	108.328384	108.449920	108.769113	108.425544
A-H <sub>11</sub> C <sub>2</sub> C <sub>1</sub>	107.849972	107.929008	107.407979	106.831797	108.812968	106.903319
A-H <sub>12</sub> C <sub>3</sub> C <sub>2</sub>	110.157955	110.766089	107.831453	108.280585	108.194488	108.845052
A-H <sub>13</sub> N <sub>5</sub> C <sub>3</sub>	111.345249	105.808645	105.648142	109.471221	109.471221	109.471221
A-H <sub>14</sub> N <sub>5</sub> C <sub>3</sub>	110.967798	112.766341	106.078293	109.471221	109.471221	109.471221
A-H <sub>15</sub> N <sub>5</sub> C <sub>3</sub>	108.451755	109.962585	-	-	107.495117	109.471221
A-H <sub>16</sub> N <sub>7</sub> C <sub>1</sub>	108.44999	-	-	-	109.471221	-
A-H <sub>17</sub> O <sub>9</sub> C <sub>4</sub>	109.911446	-	-	-	-	-
A-H <sub>18</sub> N <sub>5</sub> C <sub>3</sub>	105.517048	-	-	109.471221	-	-
A-H <sub>19</sub> O <sub>18</sub> N <sub>5</sub>	111.479538	-	-	-	-	-
A-H <sub>20</sub> O <sub>18</sub> N <sub>5</sub>	134.661436	-	-	-	-	-
A-O <sub>18</sub> H <sub>14</sub> N <sub>5</sub>	170.87837	-	-	-	-	-
A-O <sub>18</sub> H <sub>13</sub> N <sub>5</sub>	54.155556	-	-	-	-	-
A-O <sub>17</sub> H <sub>19</sub> O <sub>8</sub>	-	148.66153	-	-	-	-
A-O <sub>15</sub> H <sub>17</sub> O <sub>8</sub>	-	-	143.64037	-	-	-
A-O <sub>15</sub> H <sub>16</sub> O <sub>9</sub>	-	-	145.43489	-	-	-
A-O <sub>15</sub> H <sub>16</sub> O <sub>8</sub>	-	-	-	173.16771	-	-
A-O <sub>15</sub> H <sub>16</sub> O <sub>8</sub>	-	-	-	58.18832	-	-
A-O <sub>15</sub> H <sub>16</sub> N <sub>5</sub>	-	-	-	103.67566	-	-
A-H <sub>24</sub> O <sub>23</sub> N <sub>8</sub>	-	55.107553	-	-	-	-
A-H <sub>25</sub> O <sub>23</sub> N <sub>8</sub>	-	80.299966	-	-	-	-
A-H <sub>26</sub> N <sub>8</sub> C <sub>7</sub>	-	109.471221	-	-	-	-
A-H <sub>30</sub> O <sub>28</sub> N <sub>8</sub>	110.204465	-	-	-	-	-

<sup>a</sup>  $K_{c1}$  and  $K_{c2}$ , equilibrium constants of equations;  $K_{a1}$  and  $K_{a2}$ , first and second acidic dissociation constant between the indicated atoms ( $A^\circ$ );  $D$ , dihedral angle between the indicated atoms ( $^\circ$ );  $a_0$ , bohr radius ( $A^\circ$ );  $q$ , charges (Muliken) (au);  $d$ , distance of the IHB between the indicated atoms ( $A^\circ$ );  $A$ , H-bond angle ( $^\circ$ ).

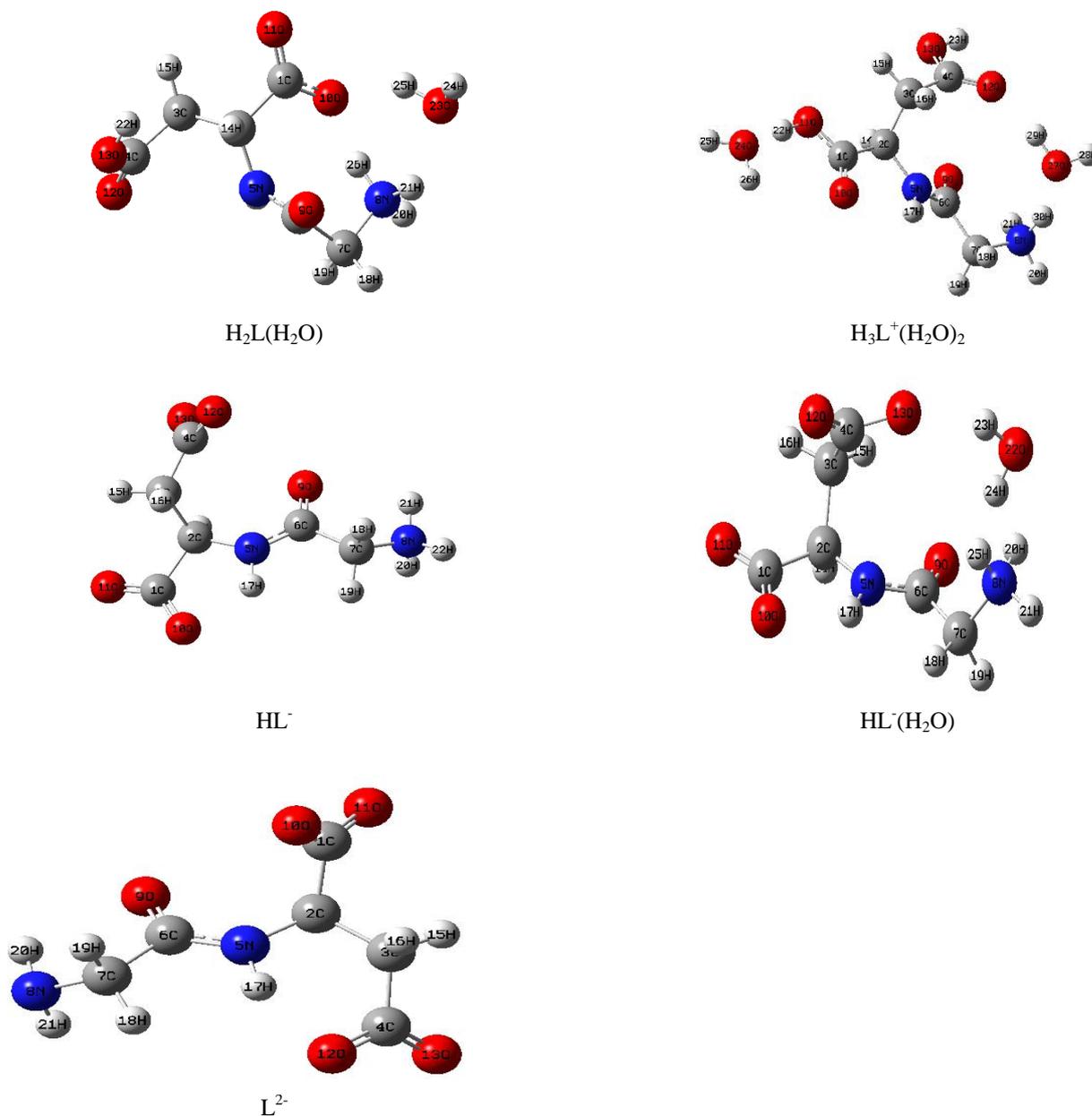


Figure 3. Calculated structure for glycyloaspargic solvated with water molecules, at the B3LYP/6-31+G(d) level of theory and using the Tomasi's method in water at 298.15 K.

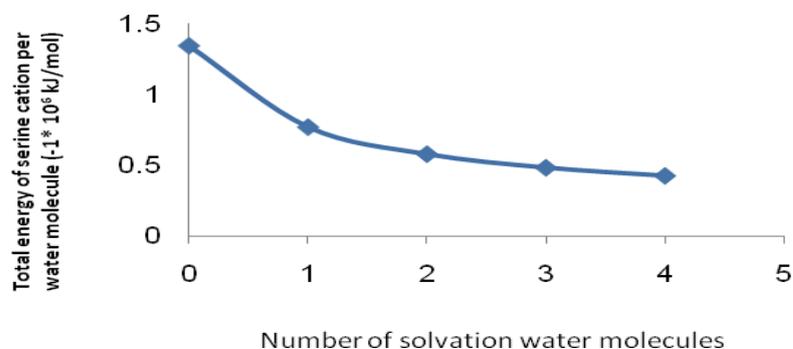
**Table 4. Calculated structural magnitudes using Tomasi's method at the B3LYP/6-31+G(d) level of theory for the cation, zwitterion molecule, and anion of glycylaspartic acid at 298.15 K<sup>a</sup>.**

Glycylaspartic acid	H <sub>3</sub> L <sup>+</sup> (H <sub>2</sub> O) <sub>2</sub>	H <sub>2</sub> L(H <sub>2</sub> O)	HL <sup>-</sup> (H <sub>2</sub> O)	HL <sup>-</sup>	L <sup>2-</sup>
K <sub>a1</sub>	0.00279298	-	-	-	-
K <sub>a2</sub>	1.60622*10 <sup>-5</sup>	-	-	-	-
K <sub>a3</sub>	4.04123*10 <sup>-9</sup>	-	-	-	-
K <sub>c1</sub>	2.77081*10 <sup>11</sup>	-	-	-	-
K <sub>c2</sub>	1593473040	-	-	-	-
K <sub>c3</sub>	68.22071138	-	-	-	-
a <sub>0</sub>	4.73	4.75	4.58	4.63	4.81
D-C <sub>4</sub> C <sub>3</sub> C <sub>2</sub> C <sub>1</sub>	-175.843906	-178.526509	79.116375	-173.286853	168.970675
D-N <sub>5</sub> C <sub>2</sub> C <sub>1</sub> C <sub>3</sub>	121.906259	123.295916	126.255312	123.027116	120.902307
D-C <sub>6</sub> N <sub>5</sub> C <sub>2</sub> C <sub>1</sub>	152.272062	70.623490	164.318993	150.735820	73.690694
D-C <sub>7</sub> C <sub>6</sub> N <sub>5</sub> C <sub>2</sub>	-179.445285	-137.158583	-108.587761	-173.686074	-179.692057
D-N <sub>8</sub> C <sub>7</sub> C <sub>6</sub> N <sub>5</sub>	-148.290007	77.074131	3.166249	154.442588	-149.321080
D-O <sub>9</sub> C <sub>6</sub> N <sub>5</sub> C <sub>2</sub>	2.246458	34.357987	122.424401	8.849814	-1.013170
DO <sub>10</sub> C <sub>1</sub> C <sub>2</sub> N <sub>5</sub>	9.852010	6.228798	-1.553488	2.588196	26.999059
DO <sub>11</sub> C <sub>1</sub> O <sub>10</sub> C <sub>2</sub>	-178.588309	179.871877	177.866544	-179.302391	-176.470065
DO <sub>12</sub> C <sub>4</sub> C <sub>3</sub> C <sub>2</sub>	-94.539567	-107.914318	-77.841686	-112.182953	-61.568262
qN <sub>5</sub>	-0.381375	-0.482010	-0.424190	-0.399092	-0.380910
qN <sub>8</sub>	-1.023477	-1.060984	-0.981593	-0.950330	-0.669608
qO <sub>9</sub>	-0.596266	0.580049	-0.694862	-0.618624	-0.680410
qO <sub>10</sub>	-0.552709	0.774108	-0.708180	-0.696917	-0.694482
qO <sub>12</sub>	-0.559503	-0.531297	-0.508321	-0.514336	-0.716632
qO <sub>13</sub>	-0.572594	-0.598840	-0.658852	-0.610212	-0.224255
qH <sub>15</sub>	0.283351	0.288106	0.262244	0.265952	-0.228838
qH <sub>20</sub>	0.531338	0.519855	0.430830	0.435027	-0.421255
qH <sub>14</sub>	0.301344	0.279871	0.261365	0.255333	-0.224255
qH <sub>16</sub>	0.276906	0.272452	0.271676	0.242266	-0.232695
qH <sub>17</sub>	0.497979	0.497333	0.491073	0.462125	-0.463805
qH <sub>19</sub>	0.301113	0.280484	0.220020	0.219999	-0.229641
d-C <sub>2</sub> C <sub>1</sub>	1.535802	1.571393	1.584357	1.586421	1.582128
d-C <sub>3</sub> C <sub>2</sub>	1.555675	1.539650	1.553571	1.546954	1.554808
d-C <sub>4</sub> C <sub>3</sub>	1.514661	1.521753	1.508607	1.505192	1.559737
d-N <sub>5</sub> C <sub>2</sub>	1.459331	1.463685	1.455981	1.457841	1.464420
d-O <sub>13</sub> H <sub>25</sub>	-	-	3.23600	-	-
d-C <sub>6</sub> N <sub>5</sub>	1.339789	1.367566	1.335989	1.346144	1.333579
d-O <sub>28</sub> H <sub>21</sub>	3.17133	-	-	-	-
d-C <sub>7</sub> C <sub>6</sub>	1.539058	1.537099	1.529543	1.541963	1.545014
d-N <sub>8</sub> C <sub>7</sub>	1.498253	-	1.472482	1.464845	1.475065
d-O <sub>9</sub> C <sub>6</sub>	1.232856	1.222142	1.248969	1.239843	1.247031
d-O <sub>10</sub> C <sub>1</sub>	1.220273	1.295784	1.261986	1.263079	1.258513
d-O <sub>9</sub> H <sub>30</sub>	3.37472	-	-	-	-
d-O <sub>11</sub> C <sub>1</sub>	1.319391	1.228287	-	1.249822	1.263646
d-H <sub>14</sub> C <sub>2</sub>	1.094271	1.093245	1.096538	1.094833	1.096603
d-N <sub>8</sub> H <sub>25</sub>	-	3.59794	-	-	-
d-H <sub>15</sub> C <sub>3</sub>	1.093135	1.095558	1.092946	1.095601	1.092472
d-H <sub>16</sub> C <sub>3</sub>	1.093579	1.0994934	1.094192	1.093648	1.100409
d-O <sub>23</sub> H <sub>21</sub>	-	-	3.59276	-	-
d-H <sub>17</sub> N <sub>5</sub>	1.018552	1.014291	1.026483	1.025641	1.029885
d-H <sub>18</sub> C <sub>7</sub>	1.091219	1.090218	1.096154	1.098559	1.095743

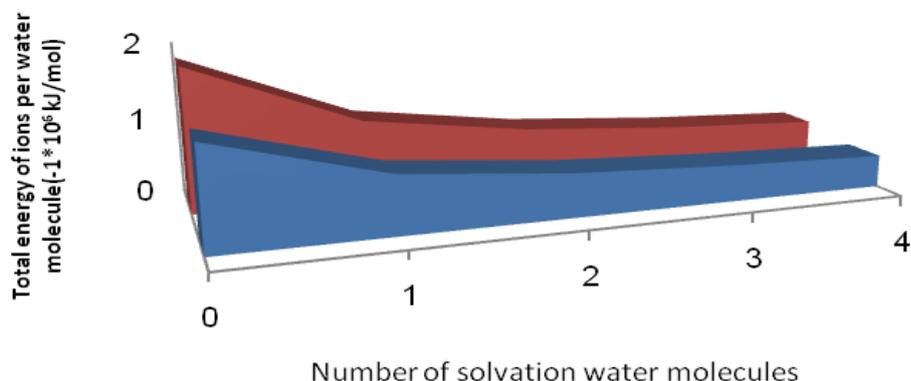
**Table 4:** Continued

d-O <sub>21</sub> O <sub>24</sub>	-	2.69133	-	-	-
d-H <sub>19</sub> C <sub>7</sub>	1.093699	1.093853	1.103435	1.096309	1.098743
d-O <sub>24</sub> H <sub>22</sub>	-	2.00462	-	-	-
d-N <sub>8</sub> H <sub>26</sub>	-	3.59794	-	-	-
d-H <sub>20</sub> N <sub>7</sub>	1.023180	1.000000	1.000000	1.000000	-
d-H <sub>21</sub> N <sub>8</sub>	1.034187	1.000000	-	1.000000	1.021605
d-H <sub>22</sub> N <sub>8</sub>	1.060506	-	-	-	-
A-C <sub>3</sub> C <sub>2</sub> C <sub>1</sub>	109.352180	109.725287	113.110683	108.676954	108.680433
A-C <sub>4</sub> C <sub>3</sub> C <sub>2</sub>	112.970254	112.960444	113.433003	114.665940	117.110915
A-O <sub>25</sub> H <sub>23</sub> O <sub>11</sub>	174.16328	-	-	-	-
A-O <sub>9</sub> H <sub>30</sub> O <sub>28</sub>	99.55132	-	-	-	-
A-N <sub>8</sub> H <sub>21</sub> H <sub>29</sub>	55.44284	-	-	-	-
A-N <sub>5</sub> C <sub>2</sub> C <sub>1</sub>	106.673443	112.095243	107.624686	107.617104	114.447386
A-C <sub>6</sub> N <sub>5</sub> C <sub>2</sub>	123.378753	119.639643	126.006337	126.043156	126.232178
A-C <sub>7</sub> C <sub>6</sub> N <sub>5</sub>	116.629089	113.688829	116.699954	115.268081	114.686018
A-O <sub>24</sub> H <sub>26</sub> O <sub>10</sub>	-	143.33234	-	-	-
A-N <sub>8</sub> C <sub>7</sub> C <sub>6</sub>	104.733575	104.297823	109.407517	114.023493	113.025947
A-O <sub>9</sub> C <sub>6</sub> N <sub>5</sub>	125.402579	124.897008	123.582757	124.850663	126.945876
A-O <sub>12</sub> C <sub>4</sub> C <sub>3</sub>	125.255070	123.912299	127.329646	126.238490	115.625665
A-O <sub>13</sub> C <sub>4</sub> C <sub>3</sub>	112.061672	116.308529	112.408151	112.476690	117.944484
A-H <sub>14</sub> C <sub>2</sub> C <sub>1</sub>	109.341590	106.426048	107.128442	108.789834	107.389443
A-H <sub>15</sub> C <sub>3</sub> C <sub>2</sub>	107.919111	107.562636	109.763498	105.968354	107.522501
A-O <sub>23</sub> H <sub>24</sub> O <sub>13</sub>	-	-	-	-	-
A-O <sub>23</sub> H <sub>25</sub> H <sub>20</sub>	-	-	157.51188	-	-
A-H <sub>16</sub> C <sub>3</sub> C <sub>2</sub>	109.446598	110.202479	106.000321	109.781110	107.264560
A-H <sub>17</sub> N <sub>5</sub> C <sub>2</sub>	114.647762	114.100655	109.747304	109.131524	112.815283
A-H <sub>18</sub> C <sub>7</sub> C <sub>6</sub>	113.783186	110.790039	110.747817	107.311182	111.373105
A-H <sub>19</sub> C <sub>7</sub> C <sub>6</sub>	110.385825	112.860309	106.316935	110.943445	107.348266
A-H <sub>20</sub> N <sub>7</sub> C <sub>7</sub>	113.305442	109.471221	109.471221	109.471221	-
A-H <sub>21</sub> N <sub>8</sub> C <sub>7</sub>	106.886330	109.471221	-	109.471221	107.433570

<sup>a</sup>  $K_{c1}$  and  $K_{c2}$ , equilibrium constants of equations;  $K_{a1}$  and  $K_{a2}$ , first and second acidic dissociation constant between the indicated atoms ( $^{\circ}$ ); D, dihedral angle between the indicated atoms ( $^{\circ}$ );  $a_0$ , bohr radius ( $^{\circ}$ );  $q$ , total atomic charges (Muliken) (au); d, distance of the IHB between the indicated atoms ( $^{\circ}$ ); A, H-bond angle( $^{\circ}$ ).



**Figure 4.** Plot of the total energy ( $\text{KJ} \cdot \text{mol}^{-1}$ ) of solvated aspartic acid per water molecule against the total number of solvation water molecules



**Figure 5.** Plot of the total energy ( $\text{KJ} \cdot \text{mol}^{-1}$ ) of solvated aspartic acid and glycyl aspartic acid cations per water molecule against the total number of solvation water molecules.

Figures 4, 5 and also, Table 1 show that when the solvation water molecules increase, the total energies of ions increase.

## CONCLUSION

In this study, the acidic dissociation constants of aspartic acid and glycylasspartic acid were calculated by Tomasi's method at the B3LYP/6-31+G(d) level of theory. To reach this goal, we determined various acid-base reactions and selected the best reaction to adapt (to match) to acidic dissociation constants ( $\text{pK}_{\text{a}1}$  and  $\text{pK}_{\text{a}2}$  and  $\text{pK}_{\text{a}3}$ ). Applying high-level ab initio method, the density functional theory (DFT) and the self-Consistent Reaction Field (SCRF) model,  $\text{pK}_{\text{a}}$  of aspartic acid and glycylasspartic acid have been successfully calculated. The obtained values were calculated with B3LYP/6-31+G(d) providing the best match with experimental values. Finally, this work express that ab initio method can be reliable to use with an acceptable degree of accuracy. In addition, it might help to develop the computational studies to obtain the properties of biomolecules.

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