THE THEORETICAL CALCULATIONS AND EXPERIMENTAL MEASUREMENTS OF ACID DISSOCIATION CONSTANT AND THERMODYNAMIC PROPERTIES OF GLYCYL-ASPARTIC ACID IN AQUEOUS SOLUTION AT DIFFERENT TEMPERATURES

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ABSTRACT

In this research work, a potentiometric technic was used to measure the acidic dissociate constants (pK_a,s) for glycyl aspartic acid (GLY-ASP) at temperatures (298.15, 303.15, 313.15, and 318.15) K and in 0.1 mol/l ionic strength of chloride sodium. Using this data, we calculated the thermodynamic properties (changes of enthalpy, ΔH, changes of entropy, ΔS, and changes of Gibbs free energy, ΔG) for acidic dissociation reaction of GLY-ASP. All analyses of data were studied in pH = 1.5-11 and in the aqueous solution. In addition, the value of the acid dissociation constants (pK_{a1}, pK_{a2}, and pK_{a3}), the optimized structure, and the thermodynamic properties of GLY-ASP were calculated in aqueous solution at various temperatures by ab initio and DFT methods. Density function theory (DFT) has been used based on the B3LYP/6-31+G(d) theory to explain the obtained acid dissociation constants of GLY-ASP as well as interactions between solvent and solvated cation, anion, and neutral species of GLY-ASP. Thomasi's method was used to analyze the formation of intermolecular hydrogen bonding between the water molecule and various species of GLY-ASP. In addition, the energy gap of anionic, cationic, and neutral species of GLY-ASP were obtained for dissociation reactions of GLY-ASP. Finally, for GLY-ASP, the theoretically calculated and experimentally determined pK_a,s were compared together and a good agreement was observed between them in the first, second, and third ionization constant of GLY-ASP.

Keywords: Glycyl aspartic acid; acid dissociation constant; thermodynamic properties; density function theory; Ab initio.

1. INTRODUCTION

Amino acids are molecules which have an amine group ($-NH_2$), and a carboxylic group ($-CO_2H$). Whenever two or more amino acids are connected together, they make a peptide. Peptides are identified from proteins based on the size [1,2]. The difference among peptides is based on the residuals of amino acids in each molecule. According to this fact, they are classified as a dipeptide, tripeptide, and so on.

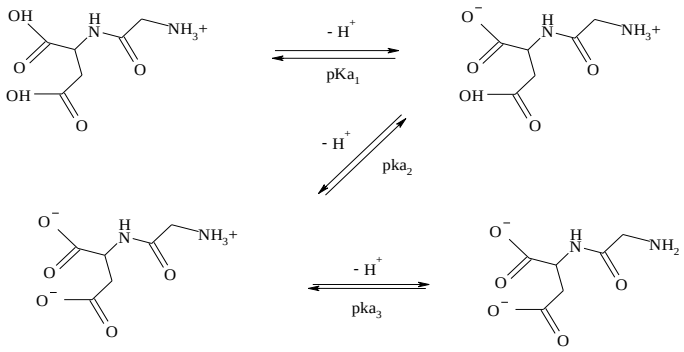

Figure 1. Suggested protonation processes of GLY-ASP.

In the last decade, the structural properties of different peptides have been investigated by many researchers. These data are used in biotechnology, medicine, drug synthesis, food supplements, and analgesic toxins [3,4]. The shortest peptides are dipeptides, including two amino acids that are joined together by one simple peptide bonding. As it can be seen in Figure 1, GLY-ASP is a dipeptide which has one carboxyl group, in low pH in acidic status, one another carboxyl group, in ordinary pH between acid and alkaline status, and an amine group in high pH in alkaline status.

Acidic dissociation constant (pK_a) for different species of amino acids and dipeptides were studied in the recent years [5,6]. The pK_a is used for determining solubility and permeability of solutions in the environmental and pharmaceutical fields [7-9]. There are various experimental techniques to measure acidic dissociation constant such as HPLC, potentiometer, and spectrophotometry [10-14]. To determine the physiochemical properties of a substance, first it must be solved in a solvent. Therefore, it is essential to measure the solubility of a substance in special solvent at different temperatures and various ionic strengths which can describe the thermodynamic system of solution, such as enthalpy and entropy changes of dissolving processes.

One of the most important physical and chemical factors of micro- and macro-molecules is the acid dissociation constant, generally known as pK_a. In the present study, the acid dissociation constant was determined for GLY-ASP, in water, by a potentiometric technique. Potentiometric technique is useful and reliable method to measure auto-proteolysis and dissociation constant of various solvents and solutions. In this technique, a glass electrode is used to measure pH and reaction potential in each step. In potentiometry, information about a sample composition is obtained through the appeared potential between two electrodes. Nowadays, selected potentiometric electrodes are used in many fields of clinical diagnosis, industrial processes control, environmental studies, and physiology. This technique is quick, cheap, and accurate [15]. In recent years, many researchers have tried to theoretically calculate the acid dissociate constant of different molecules by Ab initio and DFT methods [16,17]. Considerable research has been carried out to calculate the acid dissociation constants in the gas phase, but there is lack of research in the calculation of acidity in the solution phase [18].

The pK_a is a criterion for measuring the strength of an acid or alkaline. The pK_a equals to negative logarithm of equilibrium constant (K_a) of a neutral or charged form of a molecule by which the various species charge across various pH,s. A weak acid has a relative pK_a between 2 and 12. Acids with pK_a values lower than 2 are strong acids [19]. Acid equilibrium constants (K_a, $pK_a = -\log K_a$) are an important property of organic compounds with extensive effects on many biological and chemical systems. This parameter is an important factor in the pharmacokinetics of drugs and the interactions of proteins with other molecules [20].

In this research work, the values of the acid dissociation constant (pK_{a1}, pK_{a2}, and pK_{a3}), the thermodynamic properties (ΔH, ΔS, and ΔG) and optimized structure of GLY-ASP have been calculated in the aqueous solution at various temperatures by potentiometric, Ab initio and DFT methods. Density function theory (DFT) was used based on the B3LYP/6-31+G(d) theory to explain the obtained acid dissociation constants of GLY-ASP and also interactions between solvent and dissolved cationic, anionic, and neutral species of GLY-ASP.

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Thomasi's method was used to analyze the formation of intermolecular hydrogen bonding (IHB) between the water molecule and various species of GLY-ASP. In addition, the energy gap of anionic, cationic, and neutral species of GLY-ASP were obtained for the dissociation reactions of GLY-ASP. Finally, the ionization potential was calculated from $I = -E_{HOMO}$ while the electron affinity was determined from $A = -E_{LUMO}$ [21].

2. RESEARCH METHODOLOGY

2.1. Experimental process

2.1.1. Chemicals

GLY-ASP ($C_6H_{10}N_2O_5$) was purchased from Sigma-Aldrich. NaCl, NaOH, and HCl were purchased from Merck Company. The purity of GLY-ASP was 99%. Also, the purity of NaCl, NaOH, and HCl was 98%. These components were used without further purification. Double distilled water was used to prepare samples for this research.

2.1.2. Apparatus

The electromotive force, E, was measured using a Metrohm model 781 pH ion-meter research potentiometer equipped with a combined pH electrode which consisted of a glass electrode and a reference Ag/AgCl electrode built into a single chamber. The combined glass-pH electrode (model 6.0258.000) was modified by replacing its aqueous KCl solution with 0.01 mol.dm^{-3} NaCl and 0.09 mol.dm^{-3} $NaClO_4$ saturated with AgCl. The electrode was soaked for 15 to 20 minutes in a water–alcohol mixture before the potentiometric measurements.

2.1.3. Procedure

All titrations were carried out in an 80 cm^3 thermostated, double walled glass vessel. Potentiometric titration method (with 1M NaOH and 0.1M HCl) was used to determine the protonation constants. All tests were conducted in 0.1 mol/l ionic strength of NaCl at T = 298.15 K to 318.15 K. Analyte and titrant solutions were prepared to calculate protonation constants in the following manner:

Analyte solution: 2 ml HCl 0.1M with 2 ml NaCl 1M was reached 20 ml volume using distilled water; a certain amount of the weighted GLY-ASP was added to it later.

Alkaline titrant solution: 2 ml NaOH 1M with 2 ml NaCl 1M was reached 20 ml by distilled water.

NaCl was used for titration in certain ionic strength. The titration was done in pH = 1.5 to 11. A magnet was put in the dish for better homogenization and then the glass electrode was calibrated by the present buffers and put in the solution. After taht, we added the titrant solution to the analyte solution (0.05 to 0.05) for calibration. The calibration of the instrument was done by the Nernst eq in Excel program. An amount of the weighted GLY-ASP was added to the analyte solution and titration was continued by adding a certain amount of titrant by micropipette. Potential was read each time by the Metrohm model 781 pH ion-meter. All tests were individually conducted at T = 298.15 K to 318.15 K.

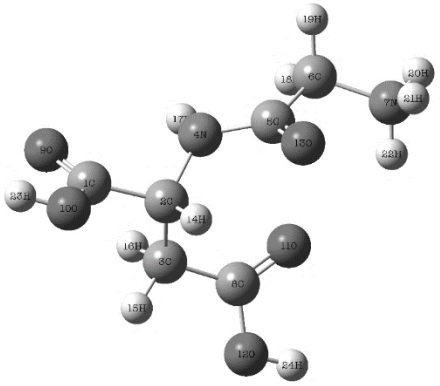


Figure 2. Optimized structure of GLY-ASP cation for performing the calculations.

2.2. Theoretical calculations

Figure 2 shows the optimized structure of cation specie of GLY-ASP. This structure was drawn by the semi-experimental PM_3 method using program Hyperchem version 8.0.8 for Windows. All calculations and optimization about studied species of GLY-ASP were done using the GAUSSIAN 98 program. DFT calculations were done using the hybrid exchange-correlation function and the Gaussian B3LYP/6-31+G(d) basis set [22-24]**.** The Polar Constellation Model (PMC) was used to analyze solvent (water) effects on all the involved samples in ionization reaction which generate hydrogen bonds with water molecules [25-27]. All reactions of various species of GLY-ASP were examined in an excel file and reactions with more errors, in pK_a values, were deleted. Finally, the suitable reactions for the first, second, and third ionization processes of GLY-ASP were selected. All calculations were carried out at T = 298.15 K to 318.15 K.

3. RESULTS AND DISCUSSION

3.1. Experimental results and discussion

In this article, the values of E'_a and K were obtained using potentiometric calibration. The electric electrodes potential, E, can be written as the eq:

$$E = E^\circ + k\log[H^+] + k\log\gamma_{H+} + E_{LJ} \quad (1)$$

In eq 1, E°, E_{LJ}, k, and γ_{H^+} show standard potentials, liquid bonding potential, Nernst slope, and proton activity coefficient, respectively.

In addition, γ_{H^+} and E_{LJ} remain in the fixed ionic strength. In this case, eq 1 can be rewritten as:

$$E = E'a - kp[H^+] \quad (2)$$

Where, E'_a is $E^\circ_{cell} + k\log\gamma_{H^+} + E_{LJ}$.

Consequently, the values of K and E'_a were calculated in calibration step using E linear regression on $[H^+]$.Results of calibration step are shown in Table 1.

Table 1. Calibration parameters of GLY-ASP in aqueous solution at temperatures 298.15 K to 318.15 K and NaCl 0.1 M.

T (K)	*E′ₐ (mV)*	*K(mV)*
298.15	410.45	59.13
303.15	416.76	59.17
308.15	421.32	59.46
313.15	426.28	59.24
318.15	431.65	59.51

Calibration parameters were used to determine concentration of hydrogen ions during titration in the second step for determining protonation constant.

Depending on pH of the solution, the GLY-ASP can exist in four different microforms which are (H_3L^+), (H_2L), (HL^-), and (L^{2-}) species. These constants are expressed by eqs 6 to 8:

$$K_1 = \frac{[H_3L^+]}{[H_2L][H^+]} \quad (3)$$

$$K_2 = \frac{[H_2L]}{[HL^-][H^+]} \quad (4)$$

$$K_3 = \frac{[HL^-]}{[L^{2-}][H^+]} \quad (5)$$

Based on Bjerrum's method, the fraction of protons bound to a ligand, $\bar{n}$, is given by eq 6 [28]:

$$\bar{n}_{cal} = \frac{C_H - [H^+]}{C_L} \quad (6)$$

where C_H and C_L are the total concentrations of protons and the GLY-ASP, respectively. Substituting, $C_L = [H_2L^+] + [H_2L] + [HL^-] + [L^{2-}]$ and $C_H = [H^+] + [HL^-] + 2[H_2L] + 3[H_3L^+]$.

Therefore, eq 6 can be rewritten as the eq 7.

$$\bar{n}_{cal} = \frac{[H_3L^+] + 2[H_2L] + 3[HL^-]}{[H_3L^+] + [H_2L] + [HL^-] + [L^{2-}]} \quad (7)$$

We can reach eq 8 using comparison eqs 3-5 and 7:

$$\bar{n}_{cal} = \frac{K_1[H^+] + 2K_1K_2[H^+]^2 + 3K_1K_2K_3[H^+]^3}{K_1[H^+] + K_1K_2[H^+]^2 + K_1K_2K_3[H^+]^3 + 1} \quad (8)$$

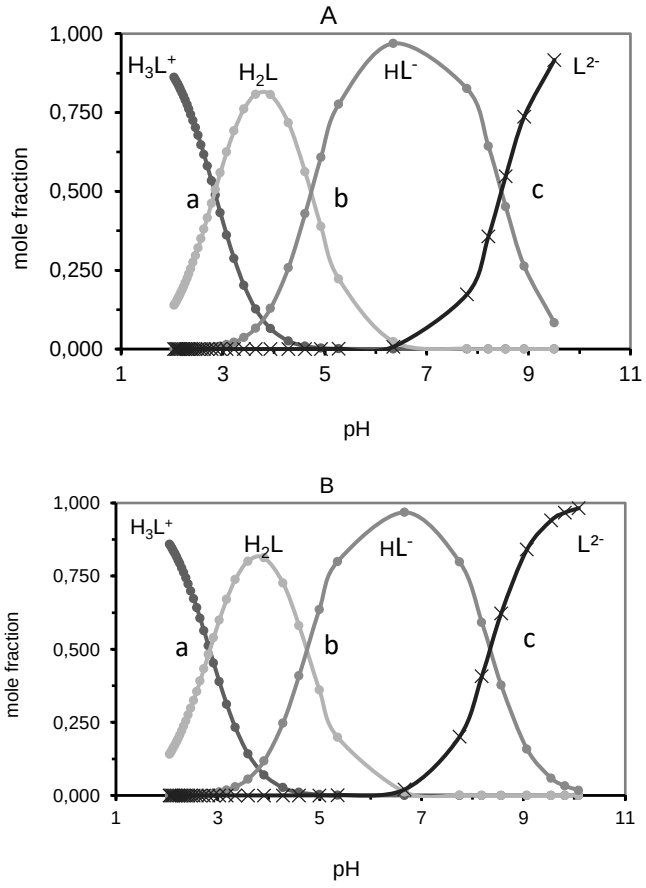
Where, K_1 and K_2 represent the protonation constants of tow carboxylic acid groups and K_3 represents the protonation constant of the amino groups of the GLY-ASP. On the other hand, electrical neutrality demands that the concentration of the cations should equal the concentration of the anions at all times during a titration, and hence:

$$\bar{n}_{exp} = \frac{C_L + [Cl^-] - [Na^+] - [H^+] + [OH^-]}{C_L} \quad (9)$$

In eq 9, $[H^+] = 10^{(E_{cell} - E^{\circ}a)/k}$ and $[OH^-]$ was determined as $K_{ap}/[H^+]$ by knowing water auto-proteolysis constant, K_{ap}, from available literature [29,30]. Finally, using a suitable computer program (Microsoft Excel Solver) [31,32] the data from eqs 8 and 9 were fitted to estimate the protonation constants of GLY-ASP in the aqueous solution at different temperatures. We used the Gauss-Newton nonlinear least-squares method in the computer program to refine the $\bar{n}$ values by minimizing the sum of error squares:

$$U = \sum (\bar{n}_{exp} - \bar{n}_{cal})^2 \quad (10)$$

Where, $\bar{n}_{exp}$ is an experimental $\bar{n}$ value and $\bar{n}_{cal}$ is the calculated one.



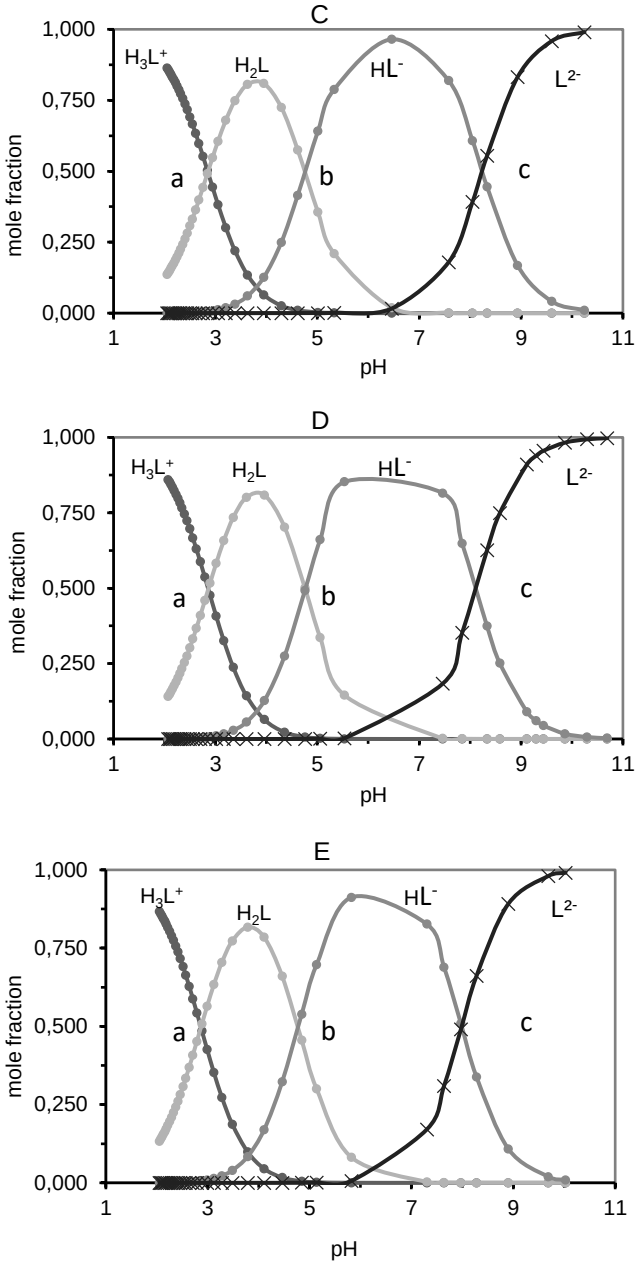


Figure3. Mole fraction diagrams obtained from titration in aqueous solution and ionic strength of 0.1M and temperatures (A) T = 298.15 K; (B) 303.15 K; (C) 308.15 K; (D) 313.15 K; (E) 318.15 K.

Figure 3 shows the mole fraction diagrams versus pH of solution for various species in the aqueous solution of GLY-ASP at different temperatures. This figure helps us find out the values of pK_a,s (pK_{a1}, pK_{a2}, and pK_{a3}) for GLY-ASP in water at various temperatures. a, b, and c are the isoelectric points in Figure 3. In these points, the concentrations of the acid and the base are equal together. For an acid (HA), the following eq shows the relationship between pK_a and pH in the aqueous solutions:

$$pK_a = pH + log\frac{[A^-]}{[HA]} \quad (11)$$

In eq 11, $[A^-]$ and [HA] are the concentrations of acid HA and base A^-. At isoelectric points (a, b, and c), $[A^-] = [HA]$ and $pH = pK_a$.

The experimentally determined pK_a,s of GLY-ASP, in aqueous solution, at various temperatures are listed in Table 2. It can be seen in this table that the experimental pKa_1 and pKa_2 increase with temperature growth during the deprotonation process of GLY-ASP while the experimental and pKa_3 decreases by temperature increasing.

Table 2. Experimental and calculated protonation constants of GLY-ASP in aqueous solution at temperatures 298.15 K to 318.15 K, in NaCl 0.1 M.

Specie	*T (K)*	pK_{a1} (Exp)	pK_{a1} (Calcu)	pK_{a2} (Exp)	pK_{a2} (Calcu)	pK_{a3} (Exp)	pK_{a3} (Calcu)
	298.15	2.83[a]	2.84	4.73[a]	4.72	8.47[a]	8.46
	303.15	2.84[b]	2.85	4.75	4.73	8.35	8.33
GLY-ASP	308.15	2.85	2.86	4.76	4.74	8.24	8.24
	313.15	2.86	2.86	4.77	4.75	8.11	8.12
	318.15	2.87	2.87	4.78	4.76	7.99	7.98

a: Ref. [32]
b: This work

Table 3. Thecalculated total free energy using Thomasi's method at the B3LYP/6-31+G(d) level of theory for cation, neutral, and anion species of GLY-ASP at 298.15 K.

Specie	G°_{sol}	$G^{\circ}_{sol/molecule}$	Specie	G°_{sol}	$G^{\circ}_{sol/molecule}$
	Hartree	Kj.mol^{-1}		Hartree	Kj.mol^{-1}
H_3L^+	-720.834.828	-1.892.551.659	HL^-	-719.920.077	-1.890.149.981
$H_3L^+(H_2O)$	-797.277.515	-1.046.625.957	$HL^-(H_2O)$	-796.358.213	-1.045.419.144
$H_3L^+(H_2O)_2$	-873.724.961	-7.646.548.883	$HL^-(H_2O)_2$	-872.806.288	-7.638.508.964
$H_3L^+(H_2O)_3$	-950.167.261	-6.236.659.761	$HL^-(H_2O)_3$	-949.248.804	-6.230.631.239
$H_3L^+(H_2O)_4$	-102.660.636	-5.390.709.479	$HL^-(H_2O)_4$	-102.570.158	-538.595.848
H_2L	-720.367.585	-1.891.324.913	L^{2-}	-7.194.688	-1.888.965.153
$H_2L(H_2O)$	-796.815.803	-1.046.019.845	$L^{2-}(H_2O)$	-795.910.757	-1.044.831.746
$H_2L(H_2O)_2$	-873.267.474	-764.254.511	$L^{2-}(H_2O)_2$	-872.352.787	-7.634.540.075
$H_2L(H_2O)_3$	-949.694.754	-6.233.558.343	$L^{2-}(H_2O)_3$	-948.800.422	-6.227.688.172
$H_2L(H_2O)_4$	-1.026.156.069	-5.388.345.001	$L^{2-}(H_2O)_4$	-1.025.243.322	-5.383.552.167
H_2O	-7.643.735	-2.006.862.432	OH^-	-7.594.893	-1.994.038.966
$2H_2O$	-1.528.798	-4.013.858.764	$OH^-(H_20)$	-15.239.712	-2.000.593.001
$3H_2O$	-229.321.345	-6.020.831.335	$OH^-(H_2O)_2$	-22.884.536	-2.002.778.117
$4H_2O$	-30.572.828	-8.026.895.221	$OH^-(H_2O)_3$	-30.522.555	-2.003.424.012
$5H_2O$	-38.218.675	-1.003.431.216	-	-	-

3.2. Theoretical results and discussion

The pK_a quantity is a molecular tendency to lose a proton (H^+). GLY-ASP loses proton from two carboxyl groups, in the first and second steps of the ionization reaction, and loses proton from the ammonium group in the third step of the ionization reaction. The microscopic ionization constants k_1, k_2, and k_3 can be applied, wherein k_1 and k_2 involve two carboxyl groups and k_3 involves the ammonium group [33].

$$k_1 = \frac{[H^+][NH_3{}^+CH_2CONHCH(COOH)CH_2COO^-]}{[NH_3{}^+CH_2CONHCH(COOH)CH_2COOH]} \quad (12)$$

$$k_2 = \frac{[H^+][NH_3{}^+CH_2CONHCH(COO^-)CH_2COO^-]}{[NH_3{}^+CH_2CONHCH(COOH)CH_2COO^-]} \quad (13)$$

$$k_3 = \frac{[H^+][NH_2CH_2CONHCH(COO^-)CH_2COO^-]}{[NH_3{}^+CH_2CONHCH(COO^-)CH_2COO^-]} \quad (14)$$

The total free energies for cation, anion, and neutral species of GLY-ASP, in water, were calculated using B3LYP/6-31+G(d) theory by Thomasi's method. Table 3 shows the values of the total free energy (G°_{sol}) for selected species of GLY-ASP at 298.15 K.

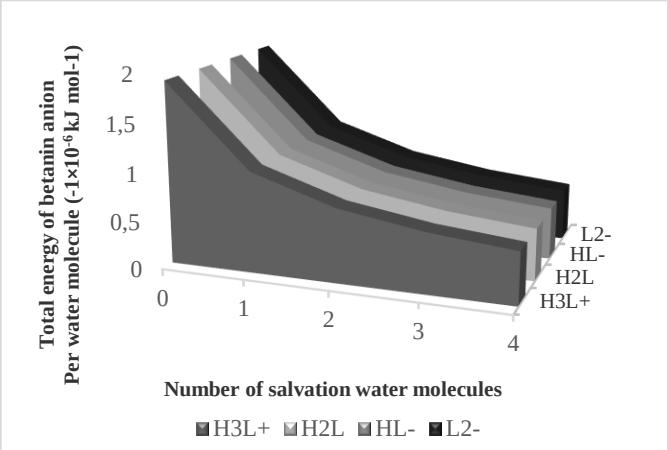


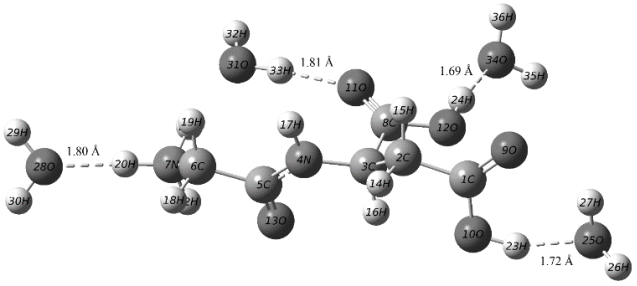
Figure 4. Plot of the total free energy (kJ mol^{-1}) of solvated species of GLY-ASP per water molecule against the total number of solvation water molecules at 298.15 K.

It can be seen in Table 3 and Figure 4 that the values of total free energy (Kj.mol^{-1}) increase for all species of GLY-ASP as the number of water molecules involved in solvation increases. This subject shows that the solvation process for all species of GLY-ASP has endothermic nature.

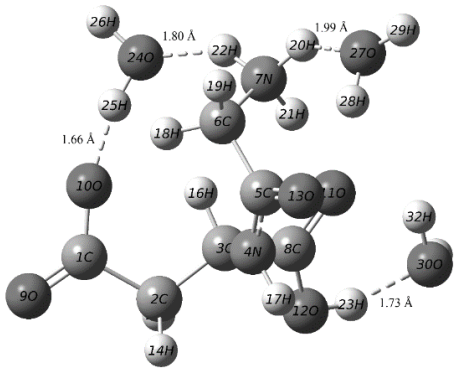
3.2.1 The first dissociation constant of the GLY-ASP

In aqueous solution, the cation specie of GLY-ASP can involve in the below reaction:

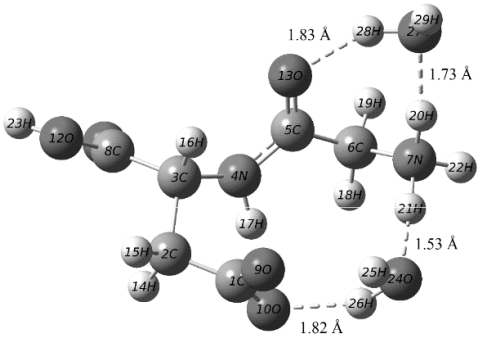
$$H_3L^+(H_2O)_4 + 0H^-(H_2O)_3 \rightleftharpoons H_2L(H_2O)_3 + 5H_2O \qquad K_{c1} \qquad (15)$$



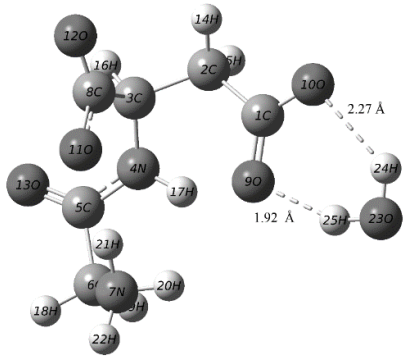
A: $H_3L^+(H_2O)_4$



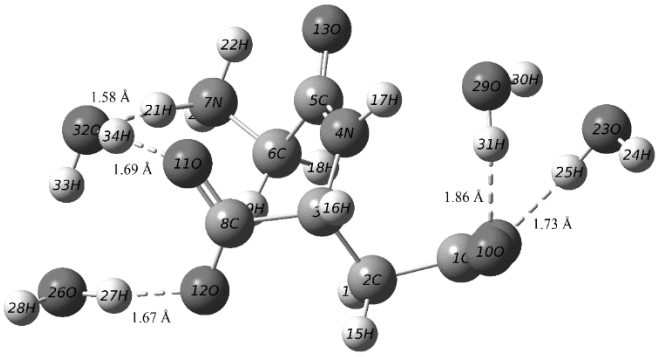
B: $H_2L(H_2O)_3$



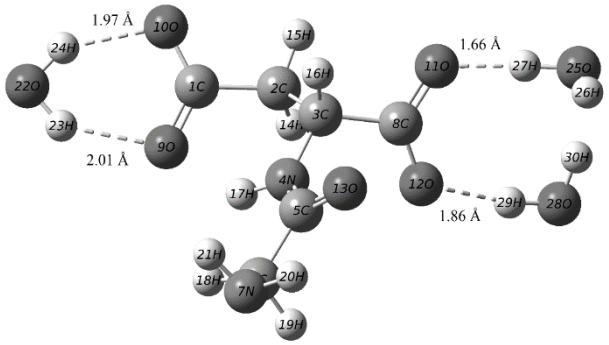
C: $H_2L(H_2O)_2$



D: $HL^-(H_2O)$



E: $HL^-(H_2O)_4$



F: $L^{2-}(H_2O)_3$

In which $H_3L^+(H_2O)_4$ (Figure5-A) and $H_2L(H_2O)_3$ (Figure5-B) show the cation species of GLY-ASP solvated with four water molecules and neutral species of GLY-ASP solvated with three water molecules, respectively. K_{c1} indicates the equilibrium constant of eq 15. This constant was theoretically calculated.

In aqueous solutions, the autoproteolysis process can happen for two, three, four, and five water molecules. In this study, the autoproteolysis process happened for five water molecules according to the below eq:

$$5H_2O \rightleftharpoons H_3O^+ + 0H^-(H_2O)_3 \qquad K_{N3} \qquad (16)$$

$$K_w = K_{N3}[H_2O]^3 \qquad (17)$$

$$K_{N3} = \frac{K_W}{[H_2O]^3} = 6.4149\times10^{-20} \qquad (18)$$

In eq 18, $K_W = 1.0081 \times 10^{-14}$ at T = 298.15 K. This shows that only a few water molecules were ionized to H^+ and OH^- ions [34].

eq 19 is obtained by combining eqs 15 and 16:

$$H_3L^+(H_2O)_4 \rightleftharpoons H_2L(H_2O)_3 + H_3O^+ \qquad K_{a1} \qquad (19)$$

It is clear that the value of constant K_{a1} can be calculated according to the following eq:

$$K_{a1} = K_{N3} \times K_{c1} \qquad (20)$$

The reaction of eq 19 shows the first ionization process of GLY-ASP. K_{a1} is applied to calculate the first acid dissociation constant (pK_{a1}) of GLY-ASP. For GLY-ASP, the calculated values of pK_{a1}, at various temperatures, are listed in Table 2. Table 2 shows that there is a good agreement between theoretically calculated and experimentally determined values of pK_{a1} for GLY-ASP at various temperatures.

Table 4 summarizes the optimized values of molecular properties for various species of GLY-ASP, in water, obtained at the B3LYP/6-31+G(d) level of theory with Tomasi's method at 298.15 K. As it can be seen in this table, the negative atomic charge of O_{10} atom (q_{O10}), in $H_2L(H_2O)_3$, increases compared to that of in $H_3L^+(H_2O)_4$ specie. It shows that the density of negative atomic charge increases in O_{10} atom during the first ionization process of GLY-ASP. This indicates that H^+ is separated from O_{10} atom in the first ionization process of GLY-ASP, (pK_{a1}).

Table 4. The calculated structural magnitudes using Thomasi's method at the B3LYP/6-31+G(d) level of theory for the cation, neutral, and anion of GLY-ASP at 298.15 K.

Calculated magnitudes	specie					
	$H_3L^+(H_2O)_4$	$H_2L(H_2O)_2$	$H_2L(H_2O)_3$	$HL^-(H_2O)$	$HL^-(H_2O)_4$	$L^{2-}(H_2O)_3$
	q: Total atomic charge (Muliken) (au)					
q_{C1}	0.651756	0.566695	0.652006	0.665395	0.335896	0.556615
q_{C2}	-0.469916	-0.572008	-0.926321	-0.658725	-0.196794	-0.441520
q_{C3}	-0.857076	-0.537964	-0.059799	-0.372012	-0.154239	-0.457014
q_{N4}	-0.287580	-0.412129	-0.458344	-0.611813	-0.470768	-0.416254
q_{C5}	0.443535	0.617521	0.569309	0.769104	0.279241	0.682373
q_{C6}	-0.199821	-0.313098	-0.200987	-0.404532	0.026503	-0.482376
q_{N7}	-1.047778	-1.158053	-1.174469	-1.036543	-1.133027	-0.944343
q_{C8}	0.913028	0.862267	0.642153	0.674438	0.195161	0.617228
q_{O9}	-0.593115	-0.681269	-0.691673	-0.798784	-0.684806	-0.747558
q_{O10}	-0.665393	-0.718271	-0.786261	-0.720063	-0.687635	-0.747719
q_{O11}	-0.609144	-0.523335	-0.559590	-0.552001	-0.687759	-0.726398
q_{O12}	-0.670961	-0.616937	-0.639145	-0.625117	-0.644641	-0.723690
q_{O13}	-0.609057	-0.671771	-0.630512	-0.647621	-0.639551	-0.687509
	d: Distance between the indicated atoms (Å)					
d_{C1-C2}	1.522524	1.54387	1.57639	1.56141	1.54276	1.54953
d_{C1-O9}	1.219051	1.25105	1.23201	1.28659	1.26692	1.27954
d_{C2-H14}	1.097349	1.09517	1.09396	1.09236	1.09405	1.09909
d_{C3-N4}	1.458984	1.46046	1.47128	1.45180	1.47906	1.46059
d_{N4-H17}	1.013753	1.02083	1.01673	1.03515	1.03124	1.02568
	A: Angles between the indicated atoms (°)					
$A_{C2-C1-O9}$	122.82867	118.32466	116.37500	117.59091	116.87168	116.03662
$A_{O9-C1-O10}$	124.75031	126.56537	128.01527	126.22749	126.54145	125.82270
$A_{C1-C2-C3}$	114.63935	107.41386	110.19841	117.94932	109.44702	115.89851
$A_{C1-C2-H14}$	106.85975	109.72167	109.43062	108.30701	110.07974	107.25619
$A_{H14-C2-H15}$	108.04726	109.6044	106.85058	107.06760	107.88843	108.33622
$A_{N4-C3-C8}$	109.87279	111.00077	106.76830	113.11783	110.12963	113.66091
$A_{N4-C3-H16}$	107.70161	107.80059	107.15360	110.38409	104.04371	108.97040
$A_{N4-C5-O13}$	124.89052	124.80483	123.00890	125.45978	126.02343	125.87278
$A_{O11-C8-O12}$	124.65657	123.25751	123.50981	120.49945	126.49487	127.57625
		D: dihedral angle between the indicated atoms (°)				
$D_{O9C1C2H14}$	-105.21793	-153.2557	-39.41902	150.62301	11.11925	56.79998
$D_{C1C2C3N4}$	164.83102	41.60976	89.76473	-48.34314	49.42594	47.48304
$D_{N4C3\ O11C8}$	-179.63306	-166.47574	112.90153	-166.82993	142.15863	37.33348
$D_{H17N4O13C5}$	162.879	-165.7033	-179.42055	-3.82956	168.25217	24.18107
$D_{O13C5C6N7}$	-35.49073	62.81359	100.89486	-124.12082	-38.33232	30.77337
$D_{H16C3N4H17}$	163.50637	140.83215	-178.50844	153.47773	20.44457	114.63236
$D_{C3N4H17C5}$	158.98503	151.43811	165.76673	154.31781	179.92230	-164.01299

3.2.2. The second ionization constant of GLY-ASP

GLY-ASP can lose the second hydrogen cation when involved in the following reaction:

$$H_2L(H_2O)_2 + 0H^-(H_2O)_3 \rightleftharpoons HL^-(H_2O) + 5H_2O \qquad K_{c2} \quad (21)$$

In which $H_2L(H_2O)_2$(Figure5-C) and $HL^-(H_2O)$ (Figure5-D) show the neutral species of GLY-ASP solvated with two water molecules and the anion species of GLY-ASP solvated with one water molecules, respectively. K_{c2} indicates the equilibrium constant of eq 21. The value of this constant was theoretically calculated.

The autoproteolysis reaction of five water molecules occurs in the second ionization process of GLY-ASP.

eq 22 is obtained by combining eqs 21 and 16:

$$H_2L(H_2O)_2 \rightleftharpoons HL^-(H_2O) + H_3O^+ \qquad K_{a2} \quad (22)$$

It is obvious that the value of the constant K_{a2} can be calculated using K_{N3} and K_{C2} according to the eq below:

$$K_{a2} = K_{N3} \times K_{c2} \qquad (23)$$

The reaction of eq 22 shows the second ionization process of GLY-ASP. K_{a2} is applied to calculate the second acid dissociation constant of GLY-ASP. For GLY-ASP, the calculated values of pK_{a2}, at various temperatures, are listed in Table 2. As it can be seen in Table 2, the theoretically calculated and experimentally determined values of pK_{a2} are very close together.

Table 4 shows that the negative value of atomic charge for the O_{12} atom (q_{O12}), in $HL^-(H_2O)$, increases compared to that of in $H_2L(H_2O)_2$.It shows that the density of negative charge increases in the O_{12} atom during the second ionization process of GLY-ASP. This subject indicates that H^+ is separated from the O_{12} atom during the second ionization process of GLY-ASP (pK_{a2}).

3.2.3. The third ionization constant of GLY-ASP

In aqueous solutions, anion specie of GLY-ASP can participate in the below reaction:

$$HL^-(H_2O)_4 + 0H^-(H_2O)_3 \rightleftharpoons L^{2-}(H_2O)_3 + 5H_2O \qquad K_{c3} \qquad (24)$$

In which $HL^-(H_2O)_4$ (Figure5-E) and $L^{2-}(H_2O)_3$ (Figure5-F) show the anion species of GLY-ASP solvated with four and three water molecules, respectively. Kc_3 indicates the equilibrium constant of eq 24. The value of this constant was theoretically calculated.

The autoproteolysis reaction of five water molecules can happen during the third ionization process of GLY-ASP.

eq 25 is obtained by combining eqs 24 and 16:

$$HL^-(H_2O)_4 \rightleftharpoons L^{2-}(H_2O)_3 + H_3O^+ \qquad K_{a3} \qquad (25)$$

It is obvious that the value of the constant K_{a3} can be calculated using K_{N3} and K_{c3} according to the below eq:

$$K_{a3} = K_{N3} \times K_{c3} \qquad (26)$$

The reaction of eq 25 shows the third ionization process of GLY-ASP. K_{a3} is applied to calculate the third acid dissociation constant of GLY-ASP. For GLY-ASP, the calculated values of pK_{a3}, at various temperatures, are listed in Table 2. As it can be seen in Table 2, the theoretically calculated value of pK_{a3} is very close to experimentally determined one at various temperatures.

Table 4 shows that the absolute value atomic charge for N_7 atom (q_{N7}), in $L^{2-}(H_2O)_3$, decreases compared to that of in $HL^-(H_2O)_4$. It shows that the density of negative charge decreases in the N_7 atom during the third ionization process of GLY-ASP. This subject indicates that H^+ is separated from the N_7 atom during the third ionization process of GLY-ASP (pK_{a3}).

For involving species in the first, second, and third ionization process of GLY-ASP, the values of total free energy were calculated at various temperatures (T = 298.15 K, 303.15 K, 308.15 K, 313.15 K, and 318.15 K) using the B3LYP/6-31+G(d) surface theory by Thomasi's method. The obtained data have been listed in Table 5.

For GLY-ASP, the values of pK_{a1}, pK_{a2}, and pK_{a3}, at various temperatures, were calculated using data of Table 5. The obtained results (pK_{a1}, pK_{a2}, and pK_{a3}, at various temperatures) were listed in Table 2. According to Table 2, the pK_{a1} and pK_{a2} increase and also, the pK_{a3} decrease with temperature growth.

4. STUDYING ON HYDROGEN BONDING

In a solution, we can find out the power of the interaction between solute and solvent molecules by calculation of distance between them (in Å). The shorter distance between molecules shows the stronger interaction between them. The water molecules which originated from the acid-base reaction and the hydration water molecule of GLY-ASP can contribute to intermolecular hydrogen bonding (IHB_s). The power of hydrogen bond is based on their length, angle, and energy as strong, medium, and weak. In strong, medium, and weak hydrogen bonds the bond lengths are 1.2 to 2.2, 1.5 to 2.2, and 2.2 to 3.2 Angstrom, respectively. Also, the bond angles in weak, moderate, and strong hydrogen bonds are 175° to 180°, 130° to 180°, and 90° to 150°, respectively [35,36]. The data of Tables 4 and Figure 5 show that all species of GLY-ASP generate moderate hydrogen bonding with water molecules. It must be noted that IHB data can be used to design and predict nano drugs. They can be conjugated to biomolecules and have a widespread application in medical science [37,38].

5. THERMODYNAMIC ANALYSIS

The changes of Gibbs free energy (ΔG), enthalpy (ΔH), and entropy (ΔS) are important thermodynamic parameters. The ΔG is the key parameter, because its value under a particular set of reactant concentrations dictates the direction of biomolecules equilibria in solutions. If its sign is negative, the binding reaction or conformational transition will proceed spontaneously to an extent governed by the magnitude of ΔG. If its sign is positive, the magnitude of ΔG specifies the energy needed to drive the reaction to form product. The free energy is a balance between enthalpy and entropy [39-41].

Change of free energy in gas or solution phases can be calculated using the below eq:

$$\Delta G = -RT \ln K_a \approx 2.303\ RT\ pK_a \qquad (30)$$

In eq 30, R is universal gas constant (8.314 K^{-1} J mol^{-1}), T is the temperature (K), and K_a is the equilibrium constant process.

The values of ΔH and ΔS can be obtained using Van't Hoff eq (by plotting ln $K_a$versus 1/T) [42]:

$$pK_a = \Delta H/2.303RT - \Delta S/2.303R \qquad (31)$$

The sign of ΔG, ΔH, and ΔS can show the state of chemical reactions. Chemical reactions can be spontaneous at each temperature when ΔG and ΔH have negative and ΔS has positive values [43,44]. The values of temperature can affect the state of chemical reactions when ΔH and ΔS have the same signs.

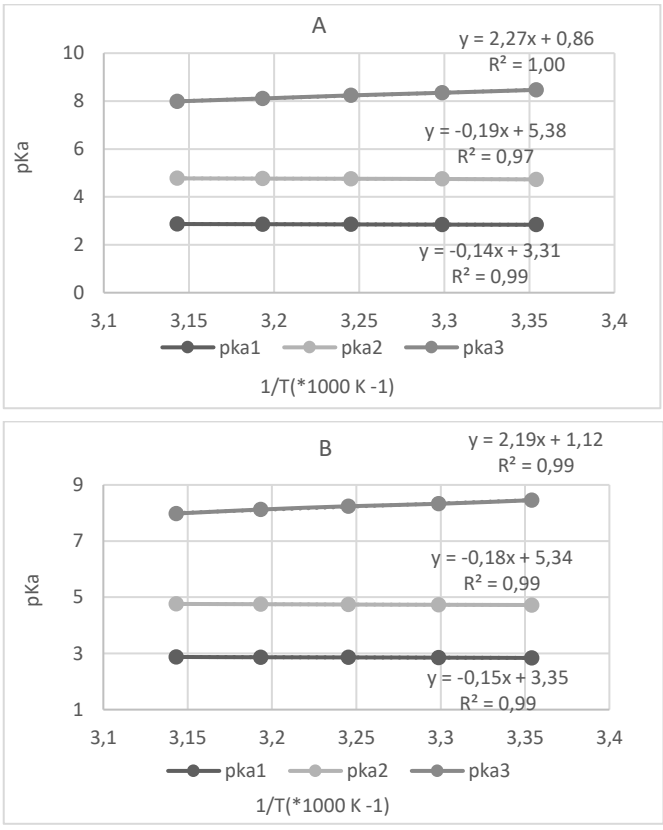
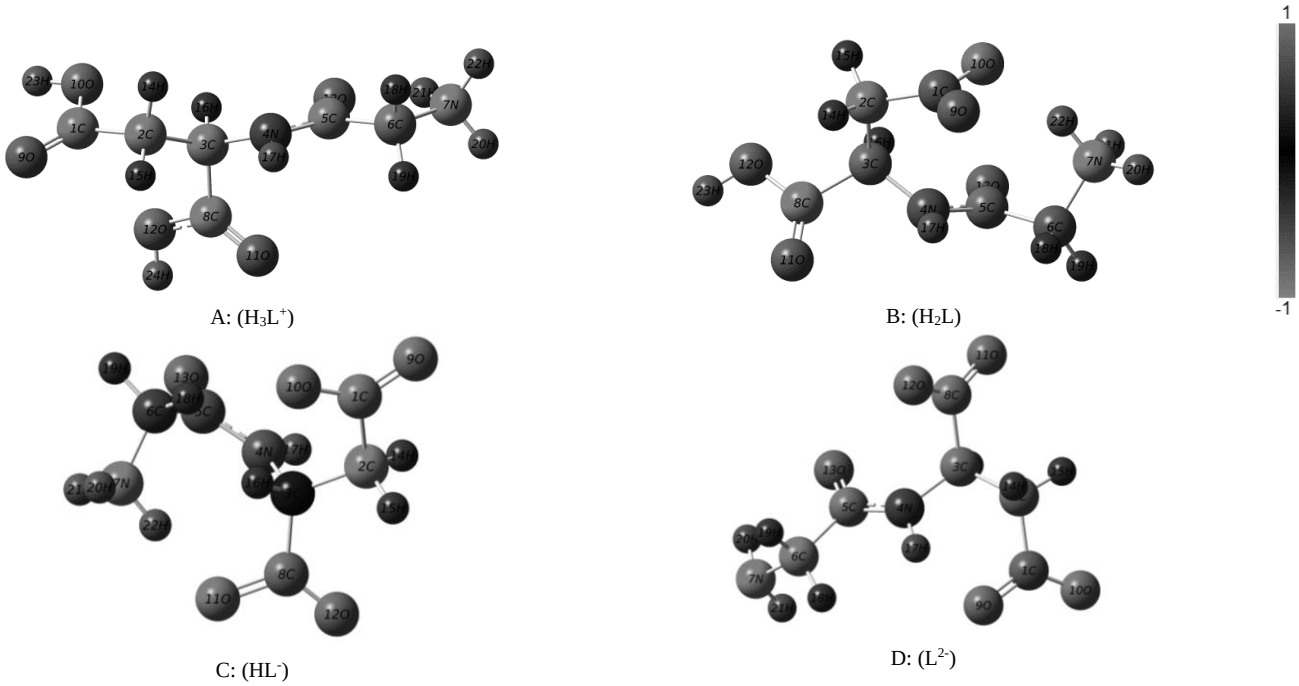


Figure 6. The plotting of calculated (A) and experimentally determined (B) pKa versus 1/T for GLY-ASP.

In order to calculate ΔH and ΔS, the pK_a values, at the different temperature T = 298.15 K, 303.15 K, 308.15 K, 313.15 K, and 318.15 K, were plotted versus 1/T by using Eq. (31) (Figure 6). The experimentally determined and theoretically calculated values of changes of Gibbs free energy, enthalpy, and entropy for GLY-ASP are listed in Table 6. According to this table, ΔG increases with temperature increase, ΔS is negative, and ΔH is negative during the first and second ionization but positive for the third ionization. As a result, the first and second ionization reactions of GLY-ASP are spontaneous at low temperature.

Table 6. The experimentally determined and theoretically calculated values of changes of Gibbs free energy, enthalpy, and entropy for GLY-ASP

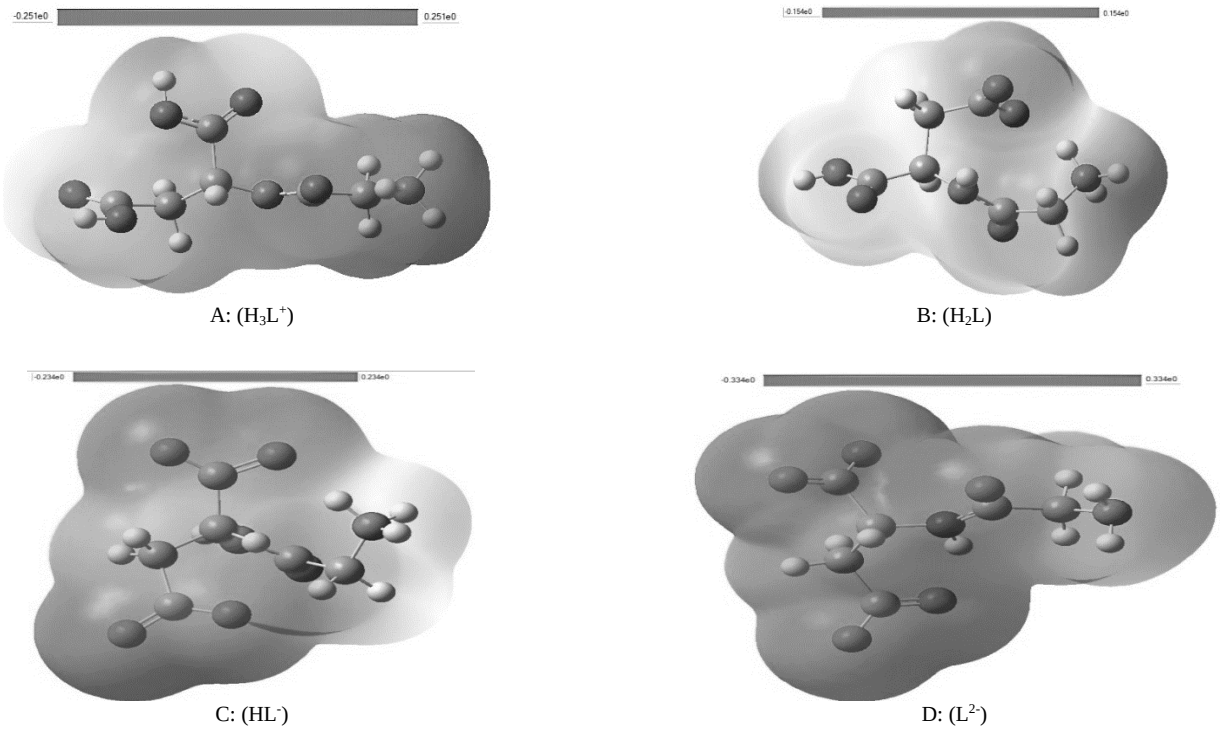
Specie	ΔH (kJ/mol)	ΔS (J/mol.K)	ΔG (kJ/mol)				
			298.15 K	303.15 K	308.15 K	313.15 K	318.15 K
GLY-ASP	**Exp.**						
	-2.73	-63.45	16.18	16.50	16.82	17.14	17.45
	-3.68	-102.96	27.01	27.53	28.04	28.56	29.07
	43.47	-16.37	48.35	48.44	48.52	48.60	48.68
	Cal.						
	-2.89	-64.1	16.22	16.54	16.86	17.18	17.50
	-3.54	-102.28	26.95	27.46	*27.98*	28.49	29.00
	41.86	-21.52	48.28	48.39	48.49	48.60	48.71



A: (H_3L^+) B: (H_2L)

C: (HL^-) D: (L^{2-})

Figure 7. The natural atomic charge distribution for different species of GLY-ASP at T = 298.15 K (cation, neutral, anion) with color range.



A: (H_3L^+) B: (H_2L)

C: (HL^-) D: (L^{2-})

Figure 8. The total electron density isosurface mapped with the molecular electrostatic potential (MEP) for different species of GLY-ASP at T = 298.15 K. (red: O; blue: N; gray: C; white: H).

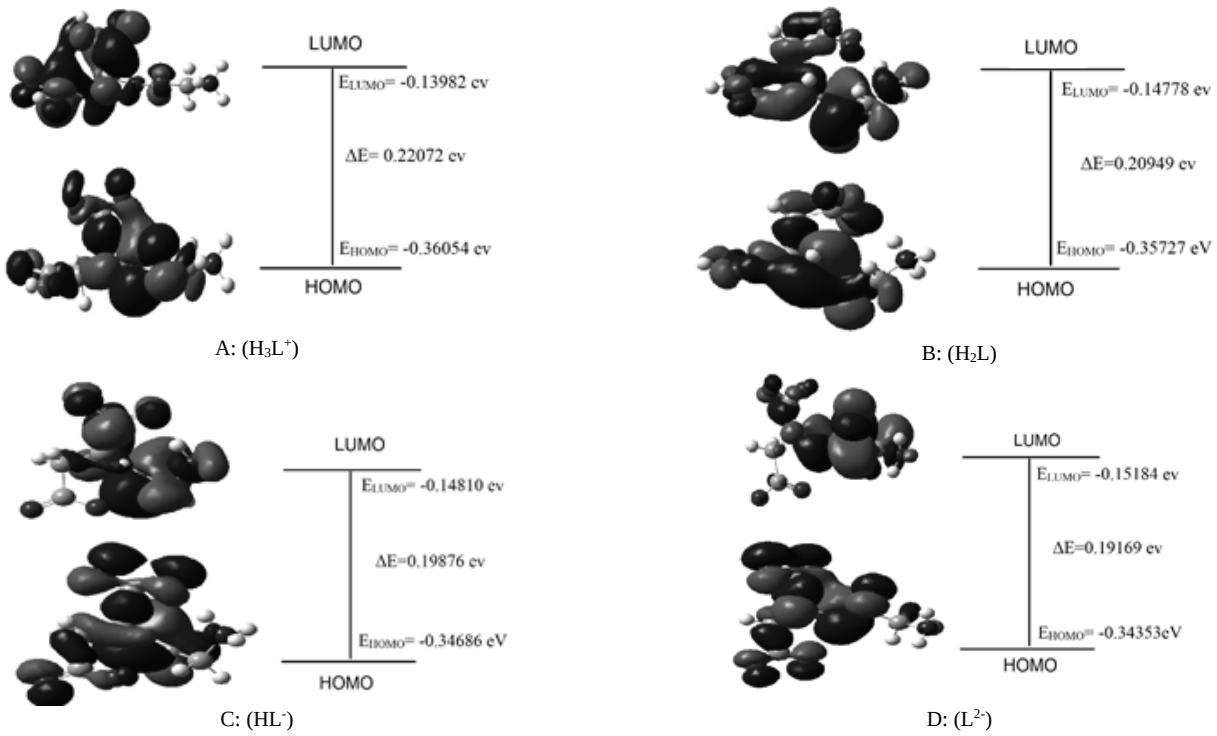


Figure 9. The atomic orbital compositions of the frontier molecular orbitals for different species of GLY-ASP at T = 298.15 K. (cationic, neutral, anionic) (red: O; blue: N; gray: C; white: H) (color figure online).

6. MULLIKEN ATOMIC CHARGES

Flux distribution is one of the important factors in molecules. Figure 7 shows the flux distribution of all GLY-ASP species at 298.15 K. It shows that the atoms C_1, C_5, and C_8 have more positive charge among carbon atoms of various species of GLY-ASP. As can be seen in Figure 7, the oxygen atoms are attached to C_1, C_5, and C_8. It is well known that the oxygen atom has high electronegativity. In addition, the atoms N_7, O_{10}, and O_{12}, compared to other atoms, have more negative charge.

Figure 8 shows the electrostatic-molecular potential distribution of molecular (MEP) charge in 3D for different species of GLY-ASP at T = 298.15 K. The charged areas of the molecule can also be seen in this figure. In a molecule, the information obtained from charge distribution can be applied to describe the reaction between various species. MEP has resulted in the correlation of electronic charges of nuclear and electrons of molecules. Therefore, it gives us useful data to detect various species [45]. MEP has a specific role in the determination of charged molecule areas with neighboring molecules. Actually, these reactive sites are useful in prediction of the reaction between one electrophile and one nucleophile. Various areas of MEP are detected with colors like red, orange, yellow, green, and blue [46]. The negative values (red) in MEP are related to the reactivity of electrophile and the positive areas (blue) are related to nucleophile reactivity [46]. There are several sites for electrophiles to attack oxygen atom in GLY-ASP. In addition, areas with positive charge are usually placed on hydrogen atom. This shows the probability of nucleophile attack on these sites.

7. HOMO AND LUMO

Energy gap is the gap between the highest full level in the HOMO (or half-occupied molecular orbital) capacity bar and the lowest empty level in the LUMO conduction bar. HOMO and LUMO are the most important orbitals in a molecule. They are called border molecular orbitals. Examination of border molecular orbitals has a determining role in the chemical stability of molecules. HOMO and LUMO energy estimate the reduction or oxidation characteristics of a molecule [48]. The energy gap between HOMO and LUMO determines the optical reactivity and chemical hardness and softness of the molecule. Energy gap or energy difference between HOMO and LUMO levels is an important stability index for structures. The high difference between these two levels (HOMO and LUMO) shows a high stability. Also, the smaller HOMO-LUMO energy gap indicates that the molecule is the more reactive and more polarizable [49]. High stability of a molecule means low reactivity in chemical reactions.

Based on the provided data in Table 7 (The values of ΔE and E_{Gap}), and according to the energy difference between species, it can be stated that the specie L^{2-} has high reactivity. According to Figure 9, HOMO focuses more on carboxyl group and LUMO focuses on the NH_3 of a molecule. It can be seen in Table 7 that differences of energy level (ΔE) for GLY-ASP decrease with increasing of negative charge.

Parameters	H_3L^+	H_2L	HL^-	L^{2-}
E_{LUMO}	-0.14	-0.15	-0.15	-0.15
E_{HOMO}	-0.36	-0.36	-0.35	-0.34
A	0.14	0.15	0.15	0.15
I	0.36	0.367	0.35	0.34
ΔE	0.22	0.21	0.20	0.19
E_{Gap}	6.01	5.70	5.41	5.22

8. CONCLUSION

In this research work, the acid dissociation constants of GLY-ASP were experimentally determined and theoretically calculated at various temperatures, T = (298.15, 303.15, 308.15, 313.15, 318.15) K. In calculation section, ab initio and DFT methods were used based on the B3LYP/6-31+G(d) theory. Also, Thomasi's method was used to analyze the formation of intermolecular hydrogen bonds between the water molecule and various species of GLY-ASP. In experimental section, the potentiometric titration technique was used to obtain pK_a,s values.

We compared the experimentally determined and theoretically calculated pK_a values of GLY-ASP at different temperatures and good agreements were observed for them. It was observed that pK_{a1} and pK_{a2} increase and pK_{a3} decreases by temperature growth. The values of ΔH and ΔS were obtained using Van't Hoff eq (plotting pK_a versus 1/T). The values of ΔG were calculated using values of ΔH, ΔS, and T. The results show that ΔG values increase with temperature growth. ΔS has negative values in first, second and third processes of ionization of GLY-ASP. In addition, ΔH has negative values in first and second ionization processes and positive values for the third ionization process.

Finally, the theoretical calculations of the HOMO-LUMO gap for GLY-ASP show that the negative charges increase in lower potential.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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