F. Kiani et al. / Hacettepe J. Biol. & Chem., 2015, 43 (1), 17–32

Study on Thermophysical Properties of Arginine and Glutamic Acid in Water Using Ab Initio Methods

Ab İnitio Yöntemler Kullanılarak Sudaki Arjinin ve Glutamik Asitin Termofiziksel Özellikleri Üzerine Bir Çalışma

Research Article

Farhoush Kiani, Azam Yousefi, and Fardad Koohyar*
Islamic Azad University, Department of chemistry, Faculty of Science, Ayatollah Amoli Branch, Amol, Iran

ABSTRACT

Gibbs free energy and acidic dissociation constants are two important thermodynamic properties of molecules. A combination of ab initio with the density functional theory (DFT) and the polarized continuum model (PCM) of Tomasi's method were utilized to calculate the acidic dissociation constants of arginine and glutamic acid in water. We applied the basis set at the B3LYP/6-31+G(d) level of theory for accurate theoretical predictions of pK_a values. Furthermore, we have evaluated the molecular conformations and solute-solvent interactions of these molecules by the electronic structure theory (commonly DFT method). It was found that in alkaline aqueous solutions the cation, anion, and neutral species of arginine and glutamic acid are solvated with one, two, three, and four molecules of water, respectively. There are intermolecular hydrogen bonds between the existent species and water molecules. The atomic charges were investigated to analyze the reaction mechanism. In this study, it can be seen that there is a good correlation between experimental attained pK_a values and the theoretical computed pK_a values.

Key Words
Arginine, Glutamic Acid, DFT Method, Acidic Dissociation Constant.

ÖZET

Gibbs serbest enerji ve asidik ayrışma sabiti, moleküllerin iki önemli termodinamik özellliğidir. Ab initio'nun yoğunluk fonksiyonel teori (DFT) ile kombinasyonu ve Tomasi'nin polarize olmuş sürekli dizi modelinden (PCM) yararlanılarak sudaki arjinin ve glutamik asitin asidik ayrışma sabitleri hesaplanmıştır. pK_a değerinin teorik tahmini için B3LYP/6-31+G(d) teori seviyesi uygulaması kullanılmıştır. Ayrıca elektronik yapı teorisiyle (geleneksel DFT yöntemi) bu moleküllerin moleküler konformasyonları ve çözünen-çözücü etkileşimleri incelenmiştir. Elde edilen bulgulara gore alkali sulu çözeltilerde arjinin ve glutamik asitin katyon, anyon ve nötral türleri, sırasıyla, bir, iki, üç ve dört su molekülü ile çözünmüştür. Varolan türler ile su molekülleri arasında molekül içi hidrojen bağları vardır. Tepkime mekanizmasını analiz etmek için atomik değişimler araştırılmıştır. Bu çalışmada, deneysel pK_a değerleri ile teorik pK_a değerleri arasında iyi bir ilişki olduğu görülmektedir.

Anahtar Kelimeler
Arjinin, Glutamik asit, DFT yöntemi, Asidik ayrışma sabiti.

Article History: Received: Oct 22, 2014; Revised: Nov 27, 2014; Accepted: Nov 27, 2014; Available Online: June, 2015.

DOI: 10.15671/HJBC.20154311201

Correspondence to: F. Koohyar, Department of Chemistry, Faculty of Science, Islamic Azad University, Amol, Iran.

Tel: +98 0114 321 70 76 Fax: +98 0114 321 71 20 E-Mail: fardadkoohyar@yahoo.com

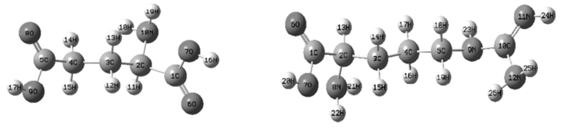
INTRODUCTION

Proteins are the primary structural and functional polymers in living systems [1]. They have a broad range of activities, including catalysis of metabolic reactions and transport of vitamins, minerals, oxygen, and fuels. Some proteins make up the structure of tissues [2-4] while others function in nerve transmission [5,6], muscle contraction and cell motility, and still others in blood clotting and immunologic defenses, and as hormones and regulatory molecules [7,8]. Proteins are synthesized as a sequence of amino acids connected together in a linear polyamide (polypeptide) structure. There are near 300 amino acids present in various animal, plant and microbial systems, but only 20 amino acids are coded by DNA to appear in proteins, such as arginine and glutamic acid [9]. These molecules are α-amino acid. It means that the α-carbon atom has a –COOH group, an $-NH_2$ group, and an 'R'-group which is responsible for the different properties of the different amino acids. Arginine and glutamic acid are classified as basic amino acids and acidic amino acids, respectively [10]. Glutamic acid contains carboxylic acid on its side-chain and is ionized at pH= 7.0. Thus, it can be carried negative charges on its α –carboxyl group. In the ionized state, this amino acid is referred to as glutamate. The side-chain of arginine is fully protonated at neutral pH and therefore, positively charged. Arginine is the most basic amino acid (pK_a = 13) and its guanidine group exists as a protonated guanidinium ion at pH= 7.0 [11].

Acid equilibrium constant (K_a, $pK_a = -\log K_a$) is an important property of organic compounds, with extensive effects on several biological and chemical systems. Aqueous pK_a values are especially useful because of their environmental and pharmacological usages [12,13].

pK_a values are related to a number of properties of drugs such as solubility, extent of binding, and rate of absorption. In addition, the determination of dosage forms and the regimes of drugs are also related to their pK_a values [14]. A number of methods (both experimental and theoretical) have been employed to calculate pK_a values [15-19]. Experimentally determined pK_a values are not always available from literature sources and often estimated values are employed in their place. Therefore, there is a high interest to expand methods for estimating pK_a of ionizable compounds and use these methods to predict the properties of a chemical in an aqueous environment [17-19]. Experimental determination of individual pK_a values can be quite complicated in complex systems such as proteins, or inhibitor-enzyme complexes. In principle, quantum chemical methods can provide a reliable and accurate means of calculating relative and (or) absolute pK_a values [20]. Theoretically, it is possible to calculate dissociation constants from first principles using quantum mechanical methods. In order to obtain accurate enough energies to calculate solution phase dissociation constants, one must account for electron correlation at the ab initio or density functional theory (DFT) and consider the effects of solvation on the molecule. In this method, electronic structure calculations were performed with density functional theory and the electrostatic features were modeled through external charge distributions and continuum dielectrics. The correlation of theoretical and experimental data can allow the development of predictive models to determine the pK_a of compounds for which no experimental data are yet available. The theoretical determination of aqueous pK_a values remains a challenging problem for computational chemists. The ab initio calculation of pK_a values in solvents (other than water) is easily achieved using continuum solvation models. Water is a very challenging solvent to model because of the large amount of hydrogen bonding that is not considered in continuum solvation models. However, such values are problematic, since the acidity of water does not allow direct equilibrium measurements of organic acids with pK_a values above the pK_a of water (15/7). The acid-base equilibria of biological important molecules are often difficult to study experimentally, particularly when the molecule contains some ionisable groups or when the pK_a of a given group is close to that of the solvent [21]. Also, potentiometric determination of accurate pK_a values below 1.5 is difficult [22].

Our purpose is to calculate the acidic dissociation constants of arginine and glutamic acid in aqueous solution by ab initio method at T = 298.15 K. To explain the obtained acidic dissociation constants, we investigated the molecular conformations (Figure 1) and solute-solvent interactions of amino acids by the electronic structure theory, (mainly) density functional theory (DFT), and self-Consistent Reaction Field (SCRF) model.



glutamic acid

arginine

Figure 1. Optimized structure of glutamic acid and arginine for performing the calculations.

COMPUTATIONAL METHOD

The optimized structures of all species of glutamic acid and arginine were carried out using DFT and ab initio methods. All the initial geometries and solvated molecules (in water) were optimized considering the intermolecular hydrogen bonds of water with amino and carboxyl groups with the Gaussian 09 program packages using hybrid density functional B3LYP [23], the Becke's three-parameter exchange functional, and the Lee-Yang–Parr correlation functional [24,25] using 6-31+G(d) basis function. All of species include cation (H_3L^+), natural (H_2L), single negative charge anion (HL^-) and double negative charge anion (L^{2-}) for glutamic acid and double negative charge cation (H_3L^{2+}), single negative charge cation (H_2L^+), natural (HL) and anion (L^-) for arginine and also, their n-hydrated((H_2O)n, n =1-4) 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) model [26].

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

RESULTS

For an acidic compound, the value of pK_a can be calculated by the following equation [27]:

$$pK_a = -1/2.303RT\ \Delta G_0 \qquad (1)$$

Where R is the gas constant, T is the temperature, and ΔG_0 is the free energy change of the dissociation reaction either in a gas or solution. The total energies of the single and solvated (in water) glutamic acid and arginine structures (cationic, natural and anionic) were calculated at the B3LYP/6-31+G(d) level of the theory, applying Tomasi's method (see Table 1).

Glutamic acid and arginine have hydrogen atoms which can contribute in interaction with the solvent molecules (water). In other words, these molecules can be ionized. Glutamic acid molecule has two carboxyl groups and one amino group while, arginine molecule has one carboxyl group and two amino groups. Therefore, these molecules have three acidic dissociation constants. It seems reasonable that at first, carboxyl and then amino group is deprotonated. We represent H_3L^+, H_2L, HL^-, and L_2^- for cationic, neutral and anionic structures of glutamic acid and also, H_3L^{+2}, H_2L^+, HL, and L^- of arginine, respectively.

DISCUSSION

First ionization constant of glutamic acid and arginine:

The following equations were chosen for the first ionization constant of glutamic acid and arginine:

$$H_3L^+(H_2O) + OH^-(H_2O) \leftrightarrow H_2L(H_2O)_2 + H_2O \quad K_{C1G} \qquad (2)$$

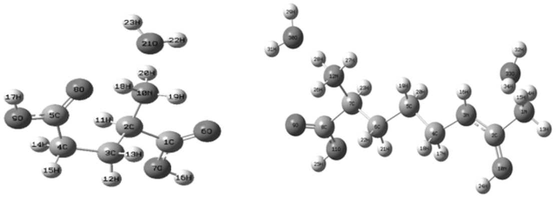
$$H_3L^+2(H_2O)_2 + OH^- \leftrightarrow H_2L^+(H_2O) + 2H_2O \quad K_{C1A} \qquad (3\ )$$

Reaction 2 is partial neutralization in alkaline glutamic acid. In this reaction, $H_3L^+(H_2O)$ is the glutamic acid cation solvated with one water molecule (Figure 2) and $H_2L(H_2O)_2$ is the neutral glutamic acid solvated with two water molecules (Figure 3). In reaction 3, H_3L^{2+} $(H_2O)_2$ (Figure 2) and $H_2L^+(H_2O)$ are the arginine cations solvated by two and one water molecule, respectively (Figure 4). These reactions are characterized by

Table 1. Calculated total energy using the Tomasi's method at the B3LYP/6-31+G(d) level of theory for cationic, neutral, and anionic species of glutamic acid and arginine at 298.15 K[a].

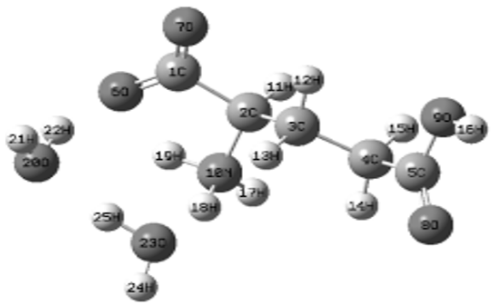
No	Solvated Species (glutamic acid)	G°sol (Hartree)	G°sol/molecule (Kj.mol^{-1})
0	H_2L	-551.683350	-1448444.496
1	$H_2L(H_2O)$	-628.126014	-824572.3457
2	$H_2L(H_2O)_2$	-704.574781	-616620.3033
3	$H_2L(H_2O)_3$	-781.020410	-512642.2224
4	$H_2L(H_2O)_4$	-857.464359	-450254.4917
0	H_3L^+	-552.133744	-1449627.006
1	$H_3L^+(H_2O)$	-628.577459	-825164.9801
2	$H_3L^+(H_2O)_2$	-705.022564	-617012.188
3	$H_3L^+(H_2O)_3$	-781.463287	-512932.9158
4	$H_3L^+(H_2O)_4$	-857.905912	-450486.3512
0	HL^-	-551.232991	-1447262.079
1	$HL^-(H_2O)$	-627.683104	-823990.9157
2	$HL^-(H_2O)_2$	-704.130309	-616231.3163
3	$HL^-(H_2O)_3$	-780.574906	-512349.8047
4	$HL^-(H_2O)_4$	-857.009304	-450015.5423
0	L_2^-	-550.767384	-1446039.628
1	$L_2^-(H_2O)$	-627.212748	-823373.4559
2	$L_2^-(H_2O)_2$	-703.653751	-615814.2486
3	$L_2^-(H_2O)_3$	-780.096824	-512036.0037
4	L_2-$(H_2O)_4$	-856.542197	-449770.2645
0	H_2L^+	-607.036550	-1593774.309
1	$H_2L^+(H_2O)$	-683.485149	-897245.0432
2	$H_2L^+ (H_2O)_2$	-759.934859	-665069.5936
3	$H_2L^+ (H_2O)_3$	-836.359913	-548965.6852
4	$H_2L^+ (H_2O)^4$	-	-
0	$H_3L^+_2$	-607.483371	-1594947.437
1	$H_3L^{+2}(H_2O)$	-	-
2	$H_3L^{+2}(H_2O)_2$	-760.374997	-665454.7877
3	$H_3L^{+2}(H_2O)_3$	-836.814652	-549264.1645
4	$H_3L^{+2}(H_2O)_4$	-913.264341	-479555.0594
0	HL	-606.576373	-1592566.114
1	$HL(H_2O)$	-683.029489	-896646.8756

2	$HL(H_2O)_2$	-759.489984	-66468.2539
3	$HL\ (H_2O)_3$	-	-
4	$HL(H_2O)_4$	-912.356261	-499078.2267
0	L^-	-606.140215	-1591420.982
1	$L^-(H_2O)$	-682.584030	-896062.0994
2	$L\text{-}(H_2O)_2$	-	
3	$L\text{-}(H_2O)_3$	-835.474892	-548384.7796
4	$L\text{-}(H_2O)_4$	-911.918515	-478848.3663
0	H_3O^+	-76.862	-201801.1616
0	H_2O	-76.434	-200677.4477
0	OH^-	-75.952	-199411.9569
2	$(H_2O)_2$	-152.87	-133786.7155
2	$2H_2O$	-152.868	-401354.8955
3	$3H_2O$	-229.302	-602032.3432
1	$OH^-(H_2O)$	-152.4	-200063.0808



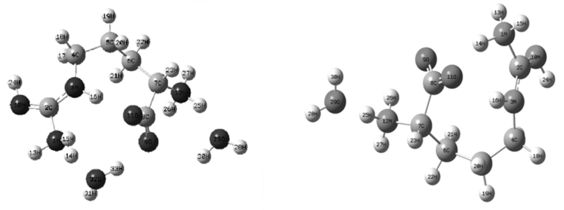
glutamic acid: $H_3L^+(H_2O)$ arginine: $H_3L^{+2}(H_2O)_2$

Figure 2. Optimized structure of glutamic acid and arginine cations solvated with one and two molecules of water, respectively and practical numbering system accepted for performing the calculation.



$H_2L(H_2O)_2$

Figure 3. Optimized structure of neutral glutamic acid solvated with two molecules of water and practical numbering system accepted for performing the calculation.



$H_2L^+(H_2O)_2$ $H_2L^+(H_2O)$

Figure 4. Optimized structure of arginine cations solvated with one and two molecules of water and practical numbering system accepted for performing the calculation.

two equilibrium constants (K_{C1G} and K_{C1A}) which were theoretically calculated. Moreover, water autopyrolysis occurs according to the following equations:

$$3H_2O \leftrightarrow H_3O^+ + OH^-(H_2O) \qquad K_w \qquad (4)$$

$$2H_2O \leftrightarrow H_3O^+ + OH^- \qquad K_N \qquad (5)$$

By merging Equations 2 and 4 for glutamic acid and also, 3 and 4 for arginine, we can obtain the Equations 6 and 7, which describe the first ionization constants of glutamic acid (K_{a1G}) and arginine (K_{a1A}):

$H_3L^+(H_2O) + 2H_2O \leftrightarrow H_2L(H_2O)_2 + H_3O^+$ K_{a1G} (6)
$H_3L + 2(H_2O)_2 \leftrightarrow H_2L^+(H_2O) + H_3O^+$ K_{a1A} (7)

It is obvious that the constants K_{C1G}, K_w, and K_{a1G} are related by:

$$K_{a1G} = K_{C1G}.K_w \quad (8)$$

Also K_{C1A}, K_N, and K_{a1A} make following equation:

$$K_{a1A} = K_{C1A}.K_N \quad (9)$$

The Equations 8 and 9 were applied to theoretical calculate the value of the first ionization constants of glutamic acid and arginine in water. Table 2 resumes the optimized values of molecular properties of the $H_3L^+(H_2O)$ cation and $H_2L(H_2O)_2$ neutral molecule (Figures 2 and 3), obtained at the B3LYP/6-31+G(d) level of theory with Tomasi's method in water at T = 298.15 K.

We have obtained the atomic charges (which are listed in Table 2) to describe the mechanism of reaction 2. This table shows that the negative atomic charge of O_7 (qO_7) of the neutral glutamic acid increases (in absolute value). It shows that the nucleophilic attack of O^- atom of the OH^- ion (see Eq 2) on the H_{16} atom of the carboxyl group bound to C_2 of the glutamic acid cation generated the neutral glutamic acid and two molecule of water (Figure 2).

In addition, in the Table 2, we are collocated the distances and angles of internal hydrogen bounds (IHBs). These values indicate that the IHB of the cation, neutral and anion of glutamic acid attach to class of weak to moderate IHBs. The properties of the moderate hydrogen bonds have the following classifications [28]: bond lengths of H• • •B are between (1.5 and 2.2) Å and the bond angle is 130° to 180°. For weak hydrogen bonds, the bond length and angle are (2.2 to 3.2) Å and 90° to 150°, respectively and also, for strong hydrogen bonds these amounts are (1.2 to 1.5) Å and 175° to 180°, respectively. As it can be seen in Table 3, the comparison of theoretical (pK_{a1}=2.19) and experimental (pK_a2=1.820) values show good range located.

For first ionization constant of arginine molecule, the same calculations and ratiocinations were done. The result of these calculations was shown in the Tables 3, 4 and also, Figure 4.

Second ionization constant of glutamic acid and arginine:
For the second ionization constant of glutamic acid, it is selected that the neutral glutamic acid solvated with two water molecules ($H_2L(H_2O)_2$) also, cationic arginine solvated by two water molecules ($H_2L^+(H_2O)_2$) according to the following reaction:

$H_2L(H_2O)_2 + OH^-(H_2O) \leftrightarrow HL^-(H_2O)_2 + 2H_2O$
K_{C2G} (9)

$H_2L^+(H_2O)_2 + OH^- \leftrightarrow \alpha HL(H_2O) + 2H_2O$
K_{C2A} (10)

In the above reactions, $HL^-(H_2O)_2$ (Figure 7) and $HL(H_2O)$ (Figure 5) display the anionic glutamic acid and neutral arginine that solvated with two and one water molecules, respectively. Equations 9 and 10 are expressed by equilibrium constants (K_{C2G} and K_{C2A}) which were theoretically calculated. The second ionization reaction of glutamic acid and arginine can be obtained by blending Equations 9 with 4 and also, equations 10 with 5:

$H_2L(H_2O)_2 + H_2O \leftrightarrow HL^-(H_2O)_2 + H_3O^+$
K_{a2G} (11)
$H_2L+(H_2O)_2 + H_2O \leftrightarrow HL(H_2O)_2 + H_3O^+$
K_{a2A} (12)

The equilibrium constants K_{a2G} and K_{a2A} that define the Equations 9 and 10 are linked with the constants K_{C2G} and K_w by eq 13, also K_{C2A} and K_N by Eq 14 for glutamic acid and arginine regularity:

$$K_{a2G} = K_{C2G}.K_w \quad (13)$$
$$K_{a2A} = K_{C2A}.K_N \quad (14)$$

Equations 13 and 14 were used to generate the values of second ionization constants of glutamic acid and arginine in water. Table 2 summarizes the determined values of the parameters and properties for the $HL^-(H_2O)_2$ anion (in water) at T = 298.15 K.

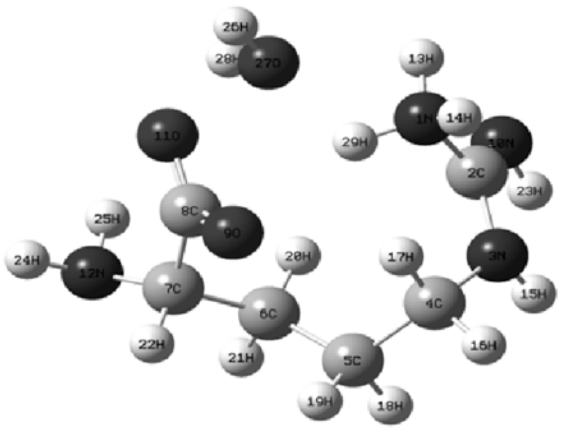
Table 2. Calculated total energy using the Tomasi's method at the B3LYP/6-31+G(d) level of theory for cationic, neutral, and anionic species of glutamic acid and arginine at 298.15 K.

glutamic acid	**$H_3L^+(H_2O)$**	**$H_2L(H_2O)_2$**	**$HL^-(H_2O)_2$**	**$HL^-(H_2O)$**	**$L_2^-(H_2O)_3$**
Ka1	0.015117671	-	-	-	-
Ka2	-	4.01355*10-5	-	-	-
Ka3	-	-	-	3.80672*10-10	-
Kc1	8.25651*1013	-	-	-	-
Kc2	-	2.192*1011	-	-	-
Kc3	-	-	-	2079039.632	-
a0	4.48	4.47	4.61	4.66	5.01
D-$C_4C_3C_2C_1$	-160.413810	-173.409047	-169.047730	-173.254723	-168.958853
D-$C_5C_4C_3C_2$	-70.975893	-179.850596	-74.796679	-78.364148	-171.463045
D-$O_6C_1C_2C_3$	-109.391427	-138.919981	-109.319073	-108.461577	67.049545
D-$O_7C_1O_6C_2$	-179.183807	179.753977	-178.928287	-179.141459	-179.013242
D-$O_8C_5C_4C_3$	21.661187	110.880933	14.718867	25.734619	-123.572594
D-$O_9C_5C_4C_3$	-159.452064	-68.525620	-166.816409	-155.636771	57.827525
D-$N_{10}C_2C_1O_6$	13.195096	-	-	-	-166.668159
D-$N_{10}C_2C_1O_7$	-	164.931461	-169.431137	-168.659662	-
D-$H_{12}C_3C_2C_1$	-41.365372	-51.970987	-48.354103	-52.302989	-47.343881
D-$H_{13}C_3C_2C_1$	73.088899	63.300306	65.311469	61.474792	69.078764
D-$H_{14}C_4C_3C_2$	51.673666	-60.674662	48.459086	44.765191	-52.187920
D-$H_{15}C_4C_3C_2$	167.004965	59.599815	162.833577	159.840647	64.679526
D-$H_{16}O_7C_1O_6$	0.696282	-	-	-	-
D-$H_{16}O_9C_5C_4$	-	178.427244	-	-	-
D-$H_{16}N_{10}C_2C_1$	-	-	103.001349	-141.575534	-124.821243
D-$H_{17}O_9C_5C_4$	-178.076824	-	-	-	-
D-$H_{17}N_{10}C_2C_1$	-	147.292738	-137.723453	-18.162038	-10.612297
D-$H_{18}N_{10}C_2C_1$	-160.627907	-85.031219	-14.930839	95.817484	-
D-$O_{18}O_8C_5C_4$	-	-	-	-	9.027223
D-$H_{19}N_{10}C_2C_1$	-40.296271	25.119467	-	-	-
D-$O_{19}O_8C_5C_4$	-	-	127.033482	-	-
D-$O_{19}O_9C_5C_4$	-	-	-	-175.949353	-
D-$H_{19}O_{18}O_8C_5$	-	-	-	-	-31.106300
D-$H_{20}N_{10}C_2C_1$	77.261591	-	-	-	-
D-$O_{20}O_6C_1O_7$	-	-79.048993	-	-	-

D-$H_{20}O_{19}O_{8}C_{5}$	-	-	136.338911	-	-
D-$H_{20}O_{19}O_{9}C_{5}$	-	-	-	178.562634	-
D-$H_{20}O_{18}O_{8}C_{5}$	-	-	-	-	64.867058
D-$O_{21}N_{10}C_{2}C_{1}$	75.731724	-	-	-	-
D-$H_{21}O_{20}O_{6}C_{1}$	-	56.863255	-	-	-
D-$H_{21}O_{19}O_{9}C_{5}$	-	-	-	-3.265913	-
D-$H_{21}O_{19}O_{8}C_{5}$	-	-	59.081118		-
D-$O_{21}O_{8}C_{5}C_{4}$	-	-	-	-	175.369269
D-$H_{22}O_{21}N_{10}C_{2}$	-90.620571	-	-		-
D-$O_{22}O_{19}O_{8}C_{5}$	-	-	-100.046249		-
D-$H_{22}O_{20}O_{6}C_{1}$	-	117.023243	-		-
D-$H_{22}O_{21}O_{8}C_{5}$	-	-	-	-	-171.681533
D-$H_{23}O_{21}N_{10}C_{2}$	114.056078	-	-		-
D-$O_{23}O_{20}O_{6}C_{1}$	-	-64.798512	-		-
D-$H_{23}O_{22}O_{19}O_{8}$	-	-	-165.569226		-
D-$H_{23}O_{21}O_{8}C_{5}$	-	-	-	-	7.040885
D-$H_{24}O_{23}O_{20}O_{6}$	-	-151.889006	-	-	-
D-$H_{24}O_{22}O_{19}O_{8}$	-	-	107.338167		-
D-$O_{24}O_{7}C_{1}O_{6}$	-	-	-	-	3.582687
D-$H_{25}O_{23}O_{20}O_{6}$	-	169.561759	-		-
D-$H_{25}O_{24}O_{7}C_{1}$	-	-	-	-	179.594284
D-$H_{26}O_{24}O_{7}C_{1}$	-	-	-	-	-2.588685
qC_{1}	0.598955	0.516163	0.521943	0.551379	0.217144
qC_{2}	0.182148	0.096157	0.166243	0.058409	0.413458
qC_{3}	-0.442762	-0.234971	-0.447585	-0.422762	-0.381924
qC_{4}	-0.721222	-0.637399	-0.660930	-0.630621	-0.622881
qC_{5}	0.709109	0.549118	0.678103	0.657450	0.607626
qO_{6}	-0.521078	-0.787485	-0717960	-0721971	-0.751263
qO_{7}	-0.611771	-0.664711	-0.709980	-0.710861	-0.771482
qO_{8}	-0.587437	-0.553721	-0.822531	-0.797741	-0.850239
qO_{9}	-0.594252	-0.625182	-0.696895	-0723281	-0.724289
qN_{10}	-1.201591	-1.165263	-1.135405	-1.053939	-1.061591
qH_{11}	0.299920	0.274519	0.251591	0.252624	0.207766
qH_{12}	0.266534	0.254494	0.237037	0.237978	0.220223
qH_{13}	0.269539	0.254471	0.238829	0.239604	0.225716
qH_{14}	0.291742	0.250626	0.250118	0.252325	0.228004

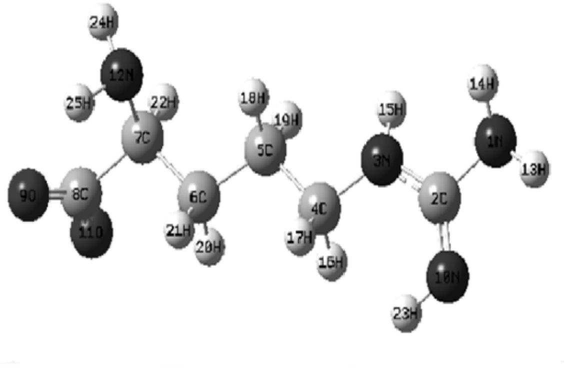
qH_{15}	0.271115	0.263698	0.237665	0.236339	0.218751
qH_{16}	0.566776	0.546382	0.565980	0.570523	0.415260
qH_{17}	0.552643	0.537330	0.570546	0.498746	0.430474
qH_{18}	0.572131	0.584406	0.509675	0.507161	-
qH_{19}	0.536366	0.528651	-	-	0.600761
qH_{20}	0.608177	-	0.537733	0.543298	0.522690
qO_{21}	-1.063751	-	-	-	-1.079859
qH_{22}	0.533844	0.601497	-	-	0.524441
qH_{23}	0.545282	-	0.606726	-	0.564019
qO_{18}	-	-	-	-	-1.150632
qO_{19}	-	-	-1.153556	-1.076855	-
qO_{20}	-	-1.141430	-	-	-
qH_{21}	-	0.540966	0.604467	0.532193	-
qO_{22}	-	-	-1.170173	-	-
qO_{23}	-	-1.125065	-	-	-
qO_{24}	-	-	-	-	-1.077078
qH_{24}	-	0.546531	0.538358	-	-
qH_{25}	-	0.590221	-	-	0.540772
qH_{26}	-	-	-	-	0.534133
d-$O_{21}H_{20}$	1.77288	-	-		-
d-O_6H_{22}	-	-	-		-
d-O_6H_{25}	-	-	-		-
d-O_6H_{22}	-	1.74913			-
d-O_6H_{25}	-	3.16989			-
d-O_8H_{21}	-	-	1.65567		-
d-$O_{22}H_{16}$	-	-	1.97016		-
d-O_9H_{20}	-	-	-	1.95300	-
d-O_8H_{21}	-	-	-	2.22183	-
d-O_7H_{25}	-	-	-		1.98302
d-O_6H_{26}	-	-	-		1.97864
d-O_8H_{23}	-	-	-		1.90629
A-$O_{21}H_{20}N_{10}$	171.82320	-	-	-	
A-$O_6H_{22}O_{20}$	-	-	-	-	
A-$O_6H_{25}O_{23}$	-	-	-	-	
A-$O_6H_{22}O_{20}$	-	159.99163	-	-	
A-$O_6H_{25}O_{23}$	-	108.20034	-	-	

K_{c1}, K_{c2} and K_{c3} equilibrium constants of equations; K_{a1}, K_{a2} and K_{a3} first, second and third acidic dissociation constant between the indicated atoms (A°); D, dihedral angle between the indicated atoms (°); aO, bohr radius (A°); q, total atomic charges (Muliken) (au); d, distance of the IHB between the indicated atoms (A°); A, H-bond angle(°).



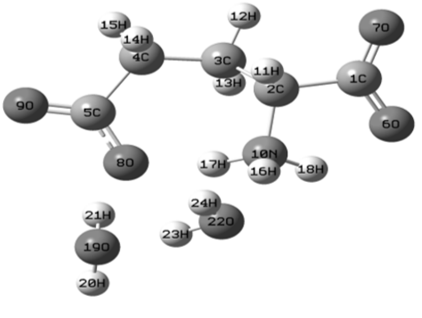
$HL(H_2O)$

Figure 5. Optimized structure of arginine natural solvated with one molecule of water and practical numbering system accepted for performing the calculation.



L^-

Figure 6. Optimized structure of arginine anion and practical numbering system accepted for performing the calculation.



$HL^-(H_2O)_2$

Figure 7. Optimized structure of glutamic acid anion solvated with two molecules of water and practical numbering system accepted for performing the calculation.

For second ionization constant of arginine molecule, the same calculations and ratiocinations were done. The result of these calculations was shown in the Tables 3, 4 and also, Figures 4 and 5.

Third ionization constant of glutamic acid and arginine:

The following equations can be chosen for the third ionization constant of glutamic acid and arginine:

$$HL^-(H_2O) + OH^-(H_2O) \leftrightarrow L_2^-(H_2O)_3 \qquad K_{C3G} \qquad (15)$$
$$HL(H_2O) + OH^-(H_2O) \leftrightarrow L^- + 3H_2O \qquad K_{C3A} \qquad (16)$$

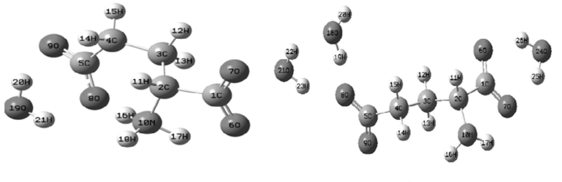
Where $HL^-(H_2O)$ and $L_2^-(H_2O)_3$ give the glutamic acid anion solvated with one and three water molecules, respectively (Figure 8). While, the $HL(H_2O)$ is neutral arginine. Equations 15 and 16 are described by equilibrium constants (K_{C3G} and K_{C3A}) which were theoretically calculated. The third ionization reactions of glutamic acid and arginine were determined by mixing Equations 15 with 4 and also, Equations 16 and 4:

$$HL^-(H_2O) + 3H_2O \leftrightarrow L_2^-(H_2O)_3 + H_3O^+ \qquad K_{a3G} \qquad (17)$$
$$HL(H_2O) \leftrightarrow L^- + H_3O^+ \qquad K_{a3A} \qquad (18)$$

The equilibrium constants Ka3G and Ka3A that define the Equations 15 and 16 are linked with constants K_{C3G}, K_{C3A} and K_w by Equations 19 and 20:

$$K_{a3G} = K_{C3G}.K_w \qquad (19)$$
$$K_{a3A} = K_{C3A}.K_w \qquad (20)$$

Equations 19 and 20 were used to obtain the value of third ionization constants of glutamic acid and arginine in water. Figure 8 represents the structure of anionic glutamic acid.



(a) $HL^-(H_2O)$ (b) $L^{2-}(H_2O)_3$

Figure 8. Optimized structure of glutamic acid anions solvated with one (a) and three molecules of water (b) and practical numbering system accepted for performing the calculation.

Table 3. Values of pK_a for the protonation of glutamic acid and arginine obtained using the Tomasi's method at the B3LYP/6-31+G(d) level of theory, at T = 298.15 K.

Species	Selected Equations	pK_a (Calculated)	pK_a (Experimental)	Ref
glutamic acid	$H_3L^+(H_2O) + 2H_2O \rightleftharpoons H_2L(H_2O)_2 + H_3O^+$	1.820515112	(I=0) 2.19	11
	$H_2L(H_2O)_2 + H_2O \rightleftharpoons HL^-(H_2O)_2 + H_3O^+$	4.396471695	(I=0) 4.25	11
	$HL^-(H_2O) + 3H_2O \rightleftharpoons L_2^-(H_2O)_3 + H_3O^+$	9.419448888	(I=0) 9.67	11
arginine	$H_3L^{+2}(H_2O)_2 \rightleftharpoons H_2L^+(H_2O) + H_3O^+$	1.953919416	(I=0) 2.17	9
	$H_2L^+(H_2O)_2 \rightleftharpoons HL(H_2O) + H_3O^+$	9.101577279	(I=0) 9.04	9
	HL(H2O) ⇌ L⁻+ H3O+	12.87493301	(I=0) 12.47	9

Table 4. 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 arginine, at T = 298.15 K.

Arginine	$H_3L^{+2}(H_2O)_2$	$H_2L^+(H_2O)$	$H_2L^+(H_2O)_2$	$HL(H_2O)$	L^-
Ka_1	0.01111938	-	-	-	-
Ka_2	-	-	7.9144*10-10	-	-
Ka_3	-	-	-	1.33373*10-13	-
Kc_1	1.10311*1012	-	-	-	-
Kc_2	-	-	78516.72716	-	-
Kc_3	-	-	-	728.4145999	-
a_0	4.85	4.84	4.72	4.89	4.71
D-$C_4N_3C_2N_1$	178.898000	158.8341968	173.8325127	30.7710921	174.2603994
D-$C_5C_4N_3C_2$	-162.643987	-130.9272835	-165.4588634	89.2525980	-177.9584861
D-$C_6C_5C_4N_3$	176.775280	71.6002917	70.3887092	-65.3840279	179.9945868
D-$C_7C_6C_5C_4$	-171.323204	-99.5149492	-109.3374092	-91.2236161	176.4593672
D-$C_8C_7C_6C_5$	170.072333	73.4404038	75.3267310	73.8980412	171.9123472
D-$O_9C_8C_7C_6$	113.710247	84.9553070	99.4680264	-82.1958164	105.0058628
D-$N_{10}C_2N_1N_3$	176.227584	-179.9544739	179.3639975	179.9543530	-179.8616652
D-$O_{11}C_8C_7C_6$	-65.8263585	-91.6718888	-77.7793921	98.3604012	-73.5598098
D-$N_{12}C_7C_6C_5$	-71.5728291	-172.1209610	-166.9971649	-161.0753724	-63.4630182
D-$H_{13}N_1C_2N_{10}$	-2.2952401	27.0384852	-12.0486420	-8.0225242	-8.2081378
D-$H_{14}N_1C_2N_{10}$	115.2301773	147.2617026	107.3819950	-128.0925242	-133.3493360
D-$H_{15}N_1C_2N_{10}$	-121.6557916	-90.8163034	-131.6423867	-	-
D-$H_{15}N_3C_2N_1$	-	-	-	178.3080210	26.5259607
D-$H_{16}N_3C_2N_1$	-31.5866697	7.5104172	2.0193057	-	-

D-$H_{16}C_4N_3C_2$	-	-	-	-150.0604048	-55.7652002
D-$H_{17}C_4N_3C_2$	-43.5626706	-9.2563803	-43.7765156	-34.9039759	61.6456598
D-$H_{18}C_4N_3C_2$	73.6653011	108.1404667	74.0672564	-	-
D-$H_{18}C_5C_4N_3$	-	-	-	56.4294593	-58.2724484
D-$H_{19}C_5C_4N_3$	-60.9050071	-168.3265858	-169.0764553	169.9438791	58.7520277
D-$H_{20}C_5C_4N_3$	55.9902170	-54.9263898	-55.2159449	-	-
D-$H_{20}C_6C_5C_4$	-	-	-	35.1013297	-62.0779743
D-$H_{21}C_6C_5C_4$	-52.7800219	25.2642864	14.8704029	151.5195225	55.7386970
D-$H_{22}C_6C_5C_4$	63.7149973	140.5515728	130.3695304	-	-
D-$H_{22}C_7C_6C_5$	-	-	-	-44.2182483	55.1487720
D-$H_{23}C_7C_6C_5$	48.1183045	-53.7812076	-49.5784408	-	-
D-$H_{23}N_{10}C_2N_1$	-	-	-	-171.2470158	-172.4908093
D-$H_{24}N_{10}C_2N_1$	177.7433970	177.8912821	-179.4555577	-	-
D-$H_{24}N_{12}C_7C_6$	-	-	-	176.6116160	154.0497483
D-$H_{25}O_{11}C_8C_7$	179.7457119	-	-	-	-
D-$H_{25}N_{12}C_7C_6$	-	157.9377475	174.1076761	-67.28605109	-96.4973424
D-$H_{26}N_{12}C_7C_6$	-69.1891299	-86.1190762	-72.2635546	-	-
D-$H_{26}O_{11}C_8C_7$	-	-	-	-178.7455807	-
D-$H_{27}N_{12}C_7C_6$	52.4756152	34.6813790	50.1936853	-	-
D-$O_{27}O_9C_8C_7$	-	-	-	169.1938476	-
D-$H_{28}N_{12}C_7C_6$	174.2845931	149.2449752	-178.0678845	-	-
D-$H_{28}O_{27}O_9C_7$	-	-	-	6.8059423	-
D-$H_{29}N_{12}C_7C_6$	166.7504103	-	-	-	-
D-$O_{29}N_{12}C_7C_6$	-	159.7839980	-171.6366529	-	-
D-$H_{29}N_1C_2N_{10}$	-	-	-	111.9074758	-
D-$O_{30}N_{12}C_7C_6$	177.3359924	-	-	-	-
D-$H_{30}O_{29}N_{12}C_7$	-	68.5127900	63.6216904	-	-
D-$H_{31}O_{30}N_{12}C_7$	81.9720628	-	-	-	-
D-$H_{31}O_9C_8C_7$	-	-	-137.4728770	-	-
D-$H_{32}N_1C_2N_{10}$	-132.4139160	-	-	-	-
-$O_{32}N_1C_2N_{10}$	-	-	110.4901754	-	-
D-$O_{33}N_1C_2N_{10}$	-124.4885541	-	-	-	-
D-$H_{33}O_{32}N_1C_2$	-	-	57.4697254	-	-
D-$H_{34}O_{33}N_1C_2$	68.4803230	-	-	-	-
qN_1	-1.124363	-1.083887	-1.121999	-0.916640	-0.874107
qC_2	0.744025	0.481182	0.687661	0.439671	0.725202

qN_3	-0.408361	-0.409269	-0.533042	-0.562246	-0.526881
qC_4	-0.707231	-0.356262	-0.351138	-0.320594	-0.743085
qO_9	-0.513122	-0.599429	-0.688013	-0.679825	-0.746462
qN_{10}	-0.765408	-0.721505	-0.791645	-0.826225	-0.924961
qO_{11}	-0.612723	-0.725265	-0.685754	-0.556453	-0.739269
qN_{12}	-1.114967	-1.230622	-1.154227	-0.924975	-0.902246
qH_{13}	0.524366	0.535502	0.521867	0.434044	0.422894
qH_{25}	0.568719	0.608284	0.582150	0.432401	0.410418
qO_{29}	-	-1.065579	-1.085201	-	-
d-$O_{33}H_{15}$	1.71797	-	-	-	-
d-$O_{30}H_{28}$	1.67694	-	-	-	-
d-O_9H_{31}	3.03170		-	-	
d-$O_{29}H_{25}$	-	1.79927	-	-	-
d-O_9H_{33}	-	-	1.73745	-	-
d-O_9H_{30}	-	-	2.42867	-	-
d-$N_{12}H_{30}$			3.05471	-	
d-$O_{11}H_{29}$	-	-	-	-	-
d-$O_{28}H_{14}$	-	-	-	-	-
d-$O_{11}H_{29}$	-	-	-	1.94665	-
d-$O_{28}H_{14}$	-	-	-	1.80519	-
d-C_2N_1	1.5051318	1.5064766	-	-	-
d-N_3C_2	1.3703879	1.3568303	-	-	-
d-C_4N_3	1.4694726	1.4639644	-	-	-
d-C_5C_4	1.5377189	1.5403606	-	-	-
d-C_6C_5	1.5426301	1.5531652	-	-	-
d-C_7C_6	1.5409427	1.5470127	-	-	-
d-C_8C_7	1.5361709	1.5650465	-	-	-
d-O_9C_8	1.2111143	1.2470301	-	-	-
d-$N_{10}C_2$	1.2612160	1.2675512	-	-	-
d-$O_{11}C_8$	1.3294308	1.2682091	-	-	-
d-$N_{12}C_7$	1.5107823	1.5195083	-	-	-
d-$H_{13}N_1$	1.0295041	1.0257840	-	-	-
d-$H_{14}N_1$	1.0281160	1.0742103	-	-	-
d-$H_{15}N_1$	1.0565020	1.0259051	-	-	-
d-$H_{16}N_3$	1.0163245	1.0186964	-	-	-
d-$H_{17}C_4$	1.0950684	1.0964933	-	-	-

d-$H_{18}C_{4}$	1.0994282	1.0963862	-	-	-
d-$H_{19}C_{5}$	1.1005630	1.0956150	-	-	-
d-$H_{20}C_{5}$	1.0972476	1.0982876	-	-	-
d-$H_{21}C_{6}$	1.0957149	1.0985253	-	-	-
d-$H_{22}C_{6}$	1.0987908	1.0988152	-	-	-
d-$H_{23}C_{7}$	1.0949070	1.0920638	-	-	-
d-$H_{24}N_{10}$	1.0188788	1.0194202	-	-	-
d-$H_{25}O_{11}$	0.9798147	-	-	-	-
d-$H_{25}N_{12}$	-	1.0159436	-	-	-
d-$H_{26}N_{12}$	1.0283826	1.0039969	-	-	-
d-$H_{27}N_{12}$	1.0239540	1.0014270	-	-	-
d-$H_{28}N_{12}$	1.0606312	3.5377629	-	-	-
d-$H_{29}N_{12}$	3.4712447	-	-	-	-
d-$O_{29}N_{12}$	-	2.8103466	-	-	-
d-$O_{30}N_{12}$	2.7298755	-	-	-	-
d-$H_{30}O_{29}$	-	0.9713305	-	-	-
d-$H_{31}O_{30}$	0.9727830	-	-	-	-
d-$H_{32}N_{1}$	3.4073810	-	-	-	-
d-$O_{33}N_{1}$	2.7678987	-	-	-	-
d-$H_{34}O_{33}$	0.9732501	-	-	-	-
A-$O_{29}H_{25}N_{12}$	-	172.98235	-	-	-
A-$O_{9}H_{33}O_{32}$	-	-	167.01077	-	-
A-$O_{9}H_{30}O_{29}$	-	-	115.98924	-	-
A-$N_{12}H_{30}O_{29}$	-	-	61.85575	-	-
A-$O_{11}H_{29}O_{28}$	-	-	-	-	-
A-$O_{28}H_{14}O_{9}$	-	-	-	-	-
A-$O_{11}H_{29}O_{28}$	-	-	-	138.53565	-
A-$O_{28}H_{14}O_{9}$	-	-	-	154.34847	-
A-$C_{3}O_{7}H_{18}$	-	-	-	-	126.01228
A-$O_{7}H_{18}O_{16}$	-	-	-	-	170.49784
A-$O_{6}H_{15}O_{13}$	-	-	-	-	173.21147

For third ionization constant of arginine molecule, the same calculations and ratiocinations were done. The result of these calculations was shown in the Tables 3, 4 and also, Figures 5 and 6.

Figure 9 shows that the total free energy increases by increasing of number of solvation water molecules. Thus, it can be seen that the solvation of species is endothermic phenomena.

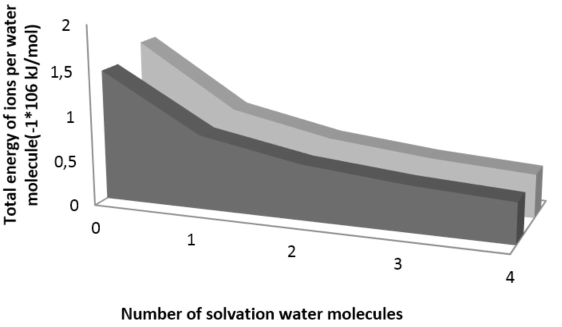


Figure 9. Plot of the total energy (KJ•mol^{-1}) of solvated glutamic acid and arginine cations per water molecule against the total number of solvation water molecules.

CONCLUSION

In the present study, different approaches are considered to develop a methodology for the accurate prediction of aqueous pK_a values of protonated glutamic acid and arginine at T = 298.15 K. We have considered different reaction schemes for approximating the acid dissociation equilibrium; one distinct equation is used for the calculation of pK_a values, and a number of levels of theory and empirical corrections are applied in the process of working toward this aim. The best correlations between the experimental and calculated data are obtained by Tomasi's method at the B3LYP/6-31+G(d)-PCM(opt) level of theory. Descriptors considered are the Gibbs free-energy change of the acid equilibrium in water, the charges on the acidic hydrogen, and on the basic nitrogen and the volume of the solvent cavity. The direct calculations differ significantly from the expected values, but the pK_a values calculated using the correlation equations are very similar and in reasonable agreement with the expected pK_a values. This research shows that ab initio method can be reliable to use with an acceptable degree of accuracy. These calculations can be useful for experimentalists and might be used for improving the acidity scale for such weak organic acids.

References

1. M.A. Meyers, P.Y. Chen, A.Y.M. Lin, Y. Seki, Biological materials: Structure and mechanical properties, Prog. Mat. Sci., 53 (2008) 1.
2. B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, Molecular Biology of the Cell. 4th ed. Garland Science, New York (2002).
3. Z.E. Sikorski, Chemical and Functional Properties of Food Proteins. Boca Raton: CRC Press., (2001) 242.
4. X. Hao, B. Sun, L. Hu, H. Lahdesmaki, V. Dunmire, Y. Feng, S.W. Zhang, H. Wang, C. Wu, H. Wang, G.N. Fuller, W.F. Symmans, I. Shmulevich, W. Zhang, Differential gene and protein expression in primary breast malignancies and their lymph node metastases as revealed by combined cDNA microarray and tissue microarray analysis, Cancer., 100 (2004) 1110.
5. H. Scott, V.M. Panin, The role of protein N-glycosylation in neural transmission, Glycobiology., 24 (2014) 407.
6. J.M. Beaulieu, R.R. Gainetdinov, The Physiology, signaling, and pharmacology of dopamine receptors, Pharmacol. Rev., 63 (2011) 182.
7. S.A. Blandin, E.A. Levashina, Phagocytosis in mosquito immune responses, Immunol. Rev., 219 (2007) 8.
8. G. Esteso, M.I. Mora, J.J. Garrido, F. Corrales, A. Moreno, Proteomic analysis of the porcine platelet proteome and alterations induced by thrombin activation, J. Proteomics., 71 (2008) 547.
9. A. Berg, T.J. Meza, M. Mahiα, T. Thorstensen, K. Kristiansen, R.B. Aalen, Ten members of the Arabidopsis gene family encoding methylαCpGαbinding domain proteins are transcriptionally active and at least one, AtMBD11, is crucial for normal development, Nucleic Acids Res., 31 (2003) 5291.
10. R.E. Wyse, E. Komor, Mechanism of aminoacid uptake by sugarcane suspension cells, Plant Physiol., 76 (1984) 865.
11. M. Zhao, H.B. Wang, L.N. Ji, Z.W. Mao, Insights into metalloenzyme microenvironments: biomimetic metal complexes with a functional second coordination sphere, Chem. Soc. Rev., 42 (2013) 8360.
12. D.W. Newton, R.B. Kluza, pK_a values of medicinal compounds in pharmacy practice, Ann. Pharmacother., 12 (1978) 546.
13. F. Kiani, R. Taherinasab, H. Tahermansouri, F. Koohyar, Determination of acidic dissociation constants of glutamine and isoleucine in water using ab initio methods, Turk. J. Biochem., 39 (2014) 503.
14. T.N. Brown, N. Mora-Diez, Computational determination of aqueous pK_a values of protonated benzimidazoles (Part 1), J. Phys. Chem. B., 110 (2006) 9270.
15. A.S. Yang, M.R. Gunner, R. Sampogna, Sharp K and Honig B. On the calculation of pK_as in proteins, Proteins: Struct., Funct., Genet., 15 (1993) 252.
16. J. Gordon, J.B. Myers, T. Folta, V. Shoja, L.S. Heath, A. Onufriev, H++: a server for estimating pK_as and adding missing hydrogens to macromolecules, Nucleic Acids Res., 33 (2005) 368.

17. A.M. Magill, B.F. Yates, An assessment of theoretical protocols for calculation of the pK_a values of the prototype imidazolium cation, Aust. J. Chem., 57 (2004) 1205.
18. J.R. Pliego, J.M. Jr Riveros, Theoretical calculation of pK_a using the cluster-continuum model., J. Phys. Chem. A., 106 (2002) 7434.
19. M. Tanaka, D. Ariga, Y. Takahash, Estimation of pK_a of selenic acid by the correlation of experimental pK_a values with those estimated by DFT calculation for inorganic oxoacids, Chem. Lett., 42 (2013) 912.
20. D. Elmali, Calculation of acidity constants of some substituted thiazole derivatives using DFT and UV spectroscopic methods, J. Art. Sci., 8 (2007) 23.
21. M. ɑmiechowski, Theoretical pK_a prediction of O-phosphoserine in aqueous solution, Chem. Phys. Lett., 501 (2010) 123.
22. E. Koort, K. Herodes, V. Pihl, I. Leito, Estimation of uncertainty in pK_a values determined by potentiometric titration, Anal. Bioanal. Chem., 379 (2004) 720.
23. M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman et al. GAUSSIAN 98, Gaussian. Inc., Pittsburgh PA (1998).
24. K. Kim, K.D. Jordan, Comparison of density functional and MP2 calculations on the water monomer and dimer, J. Phys. Chem., 98 (1994) 10089.
25. P.J. Stephens, F.J. Devlin, C.F. Chabalowski, M.J. Frisch, Ab initio calculation of vibrational absorption and circular dichroism spectra using density functional force fields, J. Phys. Chem., 98 (1994) 11623.
26. S. Miertus, E.J. Tomasi, Approximate evaluations of the electrostatic free energy and internal energy changes in solution processes, Chem. Phys., 65 (1982) 239.
27. Z.K. Jia, D.M. Du, Z.Y. Zhou, A.G. Zhang, R.V. Hou, Accurate pK_a determinations for some organic acids using an extended cluster method, Chem. Phys. Lett., 4-6 (2007) 374.
28. G.A. Jeffrey An Introduction to Hydrogen Bonding; Oxford University Press: Oxford (1997).