PROTON-LIGAND EQUILIBRIA OF ALIPHATIC, ATOXIC DIHYDRAZIDES AND WATER-SOLUBLE AMINO ACIDS GLYCINE, ASPARTIC ACID & HISTIDINE

The proton-ligand equilibria of both dihydrazides and amino acids is an essential prerequisite for the determination of formation constants of binary and ternary systems containing metal ions. It is mandatory as the formation of a metal complex is also an acid-base equilibrium, where there is a competition between the metal ion and proton for the binding site(s) of the ligand. This leads to the pH dependence of the formation of metal ligand species that can be monitored using a glass electrode. Hence, by following the changes in hydrogen ion concentration of a system containing a metal ion and ligand using Bjerrum's method of potentiometric titration and a knowledge of protonation constants of most anionic form of the ligand(s) could facilitate the speciation study of binary and ternary systems. Hence the author has taken up a study on the proton-ligand equilibria of both dihydrazides and amino acids under similar experimental conditions.

 Dihydrazides selected for the study were, succinic acid dihydrazide (SADH) and Adipic acid dihydrazide (AADH). Despite the biological importance of dihydrazides1-5 there is a paucity of information in the literature on their solution equilibria.

* **DATA ACQUISITION:**

Calvin-Wilson titration technique 6, 7 was used for the study of protonation equilibria of SADH, AADH and amino acids in aqueous medium. Requisite volumes of hydrochloric acid (to give an overall concentration of 0.02 -0.05 mol.dm-3), sodium chloride (ionic strength was maintained at 0.1 mol.dm-3) and water in the presence and absence of ligand in a total volume of 50 cm3 was titrated with ~0.2 mol. dm-3 sodium hydroxide. The concentration of the ligand was between; 0.004 to 0.015 mol.dm-3 in different experiments. Freshly prepared solutions of ligands were used in all the titrations. After the addition of each aliquot (0.1ml) of sodium hydroxide, the pH meter dial reading was recorded at regular intervals of time until two successive readings do not differ by more than 0.01 pH units. The pH correction factor and ionic product of water were calculated by Gran method 8, 9.

* **PROTON - LIGAND EQUILIBRIA OF SUCCINIC AND ADIPIC ACID DIHYDRAZIDES:**

Succinic and adipic acid dihydrazides belong to a group of nitrogenous organic compounds with general formula, R (-CO-NH-NH2)2 as shown in Figure1. In the case of aliphatic dihydrazides R= (CH2) n, the value of n= 2 for SADH and n=4 for AADH.

 

**Fig1**: General formula of a dihydrazide

In the presence of an acid the neutral form of these ligands (L) may be protonated at the terminal -NH2 groups forming mono-protonated (LH+) and biprotonated (LH22+) species. Hydrazides are also known to undergo keto-enol tautomerism and may also lose enolic protons in basic medium, leading to the formation of LH-1 and LH-2 type of deprotonated species.



**Fig2:** Keto-enol tautomerism of dihydrazides

Thus, the acido-basic equilibria of dihydrazides may contain LH22+, LH+, L, LH-1 and LH-2 type of species in solution. In literature, there are no reports on the proton-ligand equilibria of SADH. In the case of AADH, there are two reports on the protonation in aqueous-dioxane10, aqueous-dimethylformamide10 and aqueous-ethanol11 media. These authors did not consider the deprotonation of AADH in basic medium. Therefore, the author has taken up a study on the proton-ligand equilibria of these ligands which is an essential pre-requisite for the determination of the formation constants of metal-ligand complexes. Preliminary experiments revealed that, addition of a drop of 0.1 mole dm-3 alkali suddenly raised the pH of both SADH and AADH solutions to about ~ 9.0. This indicates that these ligands do not possess any dissociable protons in the pH region below 9.0.

The pH metric titration data for SADH and AADH are shown graphically in Figures3a and 4a respectively. Curve 1 represents titration of hydrochloric acid in the absence of ligand, while curve 2 represents the titration of acid + ligand with sodium hydroxide. The titration curves of both SADH and AADH (curve 2 in Figures 3a and 4a) are above that of free acid below a pH of ~5.0. This difference between the free acid and ligand curves in the lower pH region for both the ligands indicates the presence of proton associable centres in the ligands.

|  |  |
| --- | --- |
|  |  |
|  a. | 1. Titration curve of free mineral acid |  b. Species distribution diagram |
|  | 2. Titration curve for acid + SADH  |  |
| [SADH] = 0.00960 mol dm-3 |  [HCl] = 0.04039 mol dm-3  | [NaOH]= 0.2016 moldm-3 |
| Temp.: 30.0 ±0. 10C  |  Total volume= 50.0 cm3  | *I* = 0.1 mol dm-3 NaCl |
| **Figure3**: PROTON-LIGAND SYSTEM OF SADH |
|   |  |
| a. | 1. Titration curve of free mineral acid |  b. Species distribution diagram |
|  | 2. Titration curve for acid + AADH  |  |
| [AADH] = 0.0100 mol. dm-3 |  [HCl] = 0.04016 mol. dm-3 | [NaOH]= 0.2016 mol. dm-3 |
|  Temp.: 30.0 ±0. 10C  | Total volume= 50.0 cm3  | *I* = 0.1 mol. dm-3 NaCl |
| Figure 4: PROTON-LIGAND SYSTEM OF AADH |

On the basic side, i.e., above pH ~9.0, there is a significant lowering of the ligand titration curves relative to the free acid indicating deprotonation of the ligands. In between these two pH regions free acid and ligand curves coincide indicating the absence of other proton-ligand equilibria.

The titration data was first subjected to analysis by ACBA computer program12, modified by the author to run on a personal computer. The formation constants obtained from ACBA program were taken as initial estimates for refinement by MINIQUAD-75 program13. The protonation and deprotonation equilibria of SADH are shown in Figure5. The best-fit model obtained using the Miniquad-75 program (Table1) contained three formation constants β011, β012 and β01-1 corresponding to the formation of LH, LH2 and LH-1 (charges are omitted for brevity) respectively.

The species distribution diagram of SADH (Figure 3b) indicates that the LH22+ form exists only below a pH of 4.0. The extent of its formation is ~84% (at ~1.8 pH) in the pH region of study. The species LH+ which has a maximum of 62% around a pH of 3.1 ceases to exist above ~6.0 pH. β011 and β012 are the formation constants (Figure 5) of mono and biprotonated forms of SADH respectively, the protonation being at the terminal nitrogen atoms of the two hydrazide groups.



**Figure5:** PROTONATION AND DEPROTONATION

EQUILIBRIA OF SADH

**Table1:** Best fit chemical model for acido-basic equilibria of succinic acid dihydrazide in aqueous medium. Temp. = 30.0 ± 0.1°C

 and ionic strength, *I* = 0.1 mol dm–3 (NaCl)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species*mlh* | L*og β0lh (SD)* | Number of experimental points analysed | Sum of the squares of residuals, U  | χ2 |
| 011 |  3.58 (2) | 110 | 1.642 X 10-8 | 12.34 |
| 012 |  6.14 (2) |
|  01-1 | -11.91 (3) |

Up to ~99% of the ligand is in neutral form between 6.0 and 9.0 pH. In basic medium hydrazides are prone to lose a proton from the enolic form. As SADH contains two hydrazide groups, there is a probability of losing two enolic protons at higher pH. The formation constant, β01-1 in the best-fit model corresponds to the deprotonation of one of the enolic protons leading to the formation of LH-1 species. This species appears in the solution above a pH of ~9.0 and represents 49% of the total ligand at a pH of 11.5. The formation constant, β01-2 which corresponds to the deprotonation of the second enolic group leading to the formation of LH-2, was not converged. This is because its equilibrium may lie well above the pH range of the study. However, in the presence of a metal ion the ligand may also lose the second enolic proton forming both deprotonated MmL*l*H-1 and MmL*l*H-2 type of species.

 The best-fit model and the corresponding protonation and deprotonation equilibria of AADH are shown in Table 2 and Figure 6 respectively. The best-fit model for AADH (L) indicates the formation of LH22+, LH+, L and LH-1 species in aqueous medium. At lower pH (below ~3.0 pH), the biprotonated form, LH22+ of AADH dominates and with increase in pH, undergoes successive deprotonation to form the mono-protonated (LH+) and neutral species (L). β011 and β012 are the formation constants (Figure 6) of mono and biprotonated forms of AADH from its neutral form.

**Table 2:** Best fit chemical model for acido-basic equilibria of adipic acid dihydrazide in aqueous medium. Temp.= 30.0 ± 0.1°C and ionic strength, *I* = 0.1 mol dm–3 (NaCl)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species*mlh* | L*og β0lh (SD)* | Number of experimental points analysed | Sum of the squares of residuals, U  | χ2 |
| 011 |  3.67 (3) | 75 | 4.099 X 10-8 | 11.92 |
| 012 |  6.24 (7) |
|  01-1 | -12.03 (5) |

 

**Figure 6:** PROTONATION AND DEPROTONATION EQUILIBRIA

OF AADH

The relatively high values of these formation constants compared to SADH indicate the increase in basicity of the ligand with increase in chain length of “R” group connecting the two hydrazide groups. The formation constant, β01-1 in the best-fit model corresponds to the deprotonation of one of the enolic protons leading to the formation of LH-1 species. The other, β01-2 which corresponds to the formation of LH-2, is not observed as its equilibrium may lie well above the pH range of study. The species distribution diagram (Figure 4b) indicates that AADH exists in the protonated form below pH ~6.5, deprotonated form above pH ~9.0 and in neutral form between pH ~6.5 and ~9.0. The maximum percentage concentrations (with respect to total ligand) of LH22+, LH+ and L- are 85% (1.8 pH), 64%(3.1pH) and 23%(11.5 pH) respectively in the pH region of study.

### PROTON-LIGAND EQUILIBRIA OF AMINO ACIDS:

Equilibrium constants of acid- base equilibria of amino acids reported in literature were determined using different methods in various solvents and at specific conditions of temperature, ionic strength etc. Several databases are available that give the formation constants of proton-ligand and metal-ligand systems, including,

## The IUPAC Stability Constants Database, SC-Database and Mini-SC Database, 2006 and

* NIST (National Institute of Standards and Technology) -Critically Selected Stability Constants of Metal Complexes- Database Version 8.0 For Windows, R. M. Smith and A. E. Martell, (May 2004)

Along with the formation constants, these databases give the experimental conditions and methods that can be used to adjust the values for different conditions.

 Stability constants reported in the literature, mostly for amino acids and some of the other ligands, were critically surveyed and published by Martel et al.14, Pettit15, Kiss et al.16, Sovago et al.17, Berthon18and Yamauchi et al.19. Use of the values obtained either from data bases or critical surveys in speciation calculations at different experimental conditions can lead to a significant uncertainty. This may be attributed to the variations in experimental conditions and the use of classical/ graphical methods of formation constant calculations by earlier workers. Most of the classical methods ignore the possibility of formation of protonated and hydroxylated species in addition to the formation of simple mono-nuclear species. The formation constants obtained without considering these aspects are unreliable and associate with some systematic error. Also, the methods used for adjusting the equilibrium constants to the required conditions of temperature and ionic strength introduce some uncertainty in the predicted values as experienced by several workers20-22.

The protonation constants of glycine, aspartic acid and histidine, although available in literature, were, therefore, redetermined under the same experimental conditions of ionic strength and temperature used to study the binary and the ternary complexes.

### Proton-ligand equilibria of Glycine:

Glycine is the simplest possible proteinogenic non-essential amino acid with the chemical formula NH2‐CH2‐COOH. It is amphoteric in nature and in aqueous solution, depending on the pH, canexist in three different forms (Figure 7) *viz.* the cationic **(**XH2+**),** the neutral or zwitter ionic (XH) and the anionic (X-) forms. XH is the zwitter ionic form in which the amino group is protonated while the carboxyl group is deprotonated.



**Figure 7**: Protonation equilibria of glycine

The Calvin-Wilson6, 7 potentiometric titration curves obtained for glycine are shown in Figure 8a. Curve 1 represents the titration of free acid in the absence of amino acid. The titration curve for acid + amino acid (curve 2) lies above that of the free acid in the region below a pH of ~ 4.0 indicating association of a proton to the ligand. Above ~7.5 pH, i.e., in the basic region, the titration curve of ligand is well below that of the free acid, indicating the dissociation of proton from the ligand. The difference at lower pH is due to the protonation of the zwitter ionic form of the ligand at the carboxylate group and at higher pH the lowering of the curve relative to that of free acid is due to the ionization of the –NH3+ group. Therefore, the proton association and dissociation processes of the zwitter ionic form of the ligand (XH) are widely separated.

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| a. | 1. Titration curve of free mineral acid |  b. Species distribution diagram |
|  | 2. Titration curve for acid + Glycine |  |
| [Glycine] = 0.0100 mol. dm-3 |  [HCl] =0.03099 mol. dm-3 | [NaOH]= 0.2066 mol. dm-3 |
|  Temp.: 30.0 ±0. 10C  | Total volume= 50.0 cm3  | *I* = 0.1 mol. dm-3 NaCl |
| Figure 8: PROTON-LIGAND SYSTEM OF GLYCINE |

The protonation equilibria of glycine (XH) can be expressed as,

 

 The best-fit model obtained using Miniquad-75 program for glycine, is shown in Table-3. The IUPAC recommended14, 15 values for the protonation constants of glycine are 9.60 and 11.97 for *log β011* and *log β012* respectively at 25.00C and ionic strength between 0.1 and 0.2M. The observed values are in good agreement with the IUPAC and other literature values17, 18 after allowing for changes in experimental conditions.

**Table 3:** Best fit chemical model for acid-base equilibria of glycine in aqueous medium Temp. = 30.0 ± 0.1°C and ionic strength, *I* = 0.1 mol dm–3 (NaCl)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Species*Mxh* | L*og β0xh (SD)* | Literature14, 15 recommended valuesAt 25.0 0C | Number of experimental points analysed | Sum of the squares of residuals, U  | χ2 |
| 011 | 09.56 (1) | 09.60 | 84 | 3.56 X 10-8 | 6.86 |
| 012 | 11.86 (2) | 11.97 |

(SD= Standard Deviation in the least significant digit)

The constant *log β011* is due to the protonation of the amino group of the most basic form of the ligand, to form a zwitter ion. The magnitude of the protonation constant is low, when compared to the corresponding amine and may be explained as due to the electron withdrawing effect of neighbouring deprotonated carboxylate group. The second protonation constant, *log β012* is due to the addition of one more proton to the ligand at the carboxylate group. Again, the relative decrease in magnitude of the constant to that of alkyl carboxylate is due to the strong withdrawing effect of the protonated amino group. The species distribution diagram for glycine is shown in fig. 3.8b. Nearly 75% of the ligand is in biprotonated form at 1.80 pH. The zwitter ion form dominates in the intermediate region i.e., between ~4.0 and ~8.0 pH. The deprotonated form of the ligand (X-) exists only above 7.5 pH and increases monotonically above this point.

* **Proton-ligand equilibria of** **L-Histidine:**

 Among the amino acids, L-histidine is one of the strongest metal coordinating ligands which plays an important role in the binding of metal ions by proteins. Histidine has three potential metal-binding sites, namely the carboxylate oxygen, the imidazole imido nitrogen and the amino nitrogen. The imidazole nitrogen of histidine residue often provides the primary means by which the metal ions are bound to proteins.

 

**Figure 9**: L-Histidine

Histidine belongs to a group of amino acids that contain one or more amino groups in the side chain. Because amine groups can accept protons, they are bases and these amino acids are considered as basic amino acids. In solution they can accept protons from water to become positively charged. In histidine in addition to the amino group, it is the double bonded nitrogen atom that accepts the proton and has a pKa value in the physiological pH range, hence often is the only amino acid seen in biologically active sites when the donation or abstraction of a proton is needed. Structurally, it is possible that histidine (XH) can exist (Figure 10) in solution as XH32+, XH2+, XH and X-.

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| --- | --- | --- | --- |
|  |  |  |  |

**Figure 10**: Fischer Projections of successive protonated species of L-Histidine

 The first centre to protonate in the fully ionized histidinate anion (X-) is the amino-nitrogen, followed by the pyridine-like imidazole-nitrogen of the side chain and finally the carboxylate group. Since these protonation reactions take place over a widely separated and accessible pH ranges, the formation constants (Fig.11) can be determined comparatively accurately. At higher pH (>14), the pyrrole like proton may also ionize (forming an imidazolate side chain) to give a dianion (X2-)15 which is of course out of the pH region in aqueous solutions.



**Figure 11**: PROTON-LIGAND EQUILIBRIA OF L-HISTIDINE

The alkalimetric titration curves obtained, in the presence and absence of histidine are shown in Figure 12a. The titration curve for the acid+ ligand (curve 2) is above that of free acid (curve 1) up to a pH of ~ 7.0 indicating the initial association of the proton to the ligand. The wide difference between the curves indicates proton associations at more than one centre. The two buffer regions below ~7.0 pH correspond to the titrations of carboxylic and ring protons. The buffer region above pH ~7.0 indicates the titration of ammonium proton.

|  |  |
| --- | --- |
|   |  |
|  a. | 1. Titration curve of free mineral acid |  b. Species distribution diagram |
|  | 2. Titration curve for acid + Histidine |  |
| [Histidine] = 0.0100 mol dm-3 |  [HCl] = 0.02944 mol dm-3  | [NaOH]= 0.2030 moldm-3 |
| Temp.: 30.0 ±0. 10C  |  Total volume= 50.0 cm3  | *I* = 0.1 mol dm-3 NaCl |
| Figure 12: PROTON-LIGAND SYSTEM OF L-HISTIDINE |

 The titration curve for the acid+ ligand (curve 2) is above that of free acid (curve 1) up to a pH of ~ 7.0 indicating the initial association of the proton to the ligand. The wide difference between the curves indicates proton associations at more than one centre. The two buffer regions below ~7.0 pH correspond to the titrations of carboxylic and ring protons. The buffer region above pH ~7.0 indicates the titration of ammonium proton.

 The titration data of all the experiments with different concentrations of the ligand (0.004, 0.01 and 0.015 mol dm-3) were analysed by the ACBA12 computer program and the constants obtained were used as the initial estimates for the refinement using the MINIQUAD-75 program13. The best-fit chemical model thus obtained along with the statistical parameters is shown in Table 4.

**Table 4:** Best fit chemical model for acido-basic equilibria of L-histidine in aqueous medium. Temp. = 30.0 ± 0.1°C and ionic strength, *I* = 0.1 mol dm–3 (NaCl)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species*mlh* | L*og β0lh (SD)* | Number of experimental points analysed | Sum of the squares of residuals, U  | χ2 |
| 011 |  08.95 (1) | 102 | 4.021 X 10-8 | 23.12 |
| 012 |  15.02 (2) |
| 013 |  16.74 (2) |

 (SD= Standard Deviation in the least significant digit)

The results are in good agreement with the literature reports (Table 5) after allowing for the changes in experimental conditions and calculation methods.

**Table 5**: Some representative literature reports on the protonation constants of Histidine

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| S.No. | Experimental conditions | *Log β011* | *Log β012* | *Log β013* | Reference |
| Temp.0C | Ionic strength |
| 1. | 37.0 | 0.10 M (NaNO3) | 8.80 | 14.55 | 16.65 | 23 |
| 2. | 25.0 | 0*.*10 M (KCl) | 9.11 | 15.19 | 16.96 | 24 |
| 3. | 25.0 | 0.10M (KNO3) | 9.16 | 15.25 | 17.31 | 25 |
| 4. | 25.0 | 0.10M (KNO3) | 9.12 | 15.17 | 16.60 | 26 |
| 5. | 25.0 | 0.10M (KNO3) | 9.12 | 15.22 | 16.93 | 27 |
| 6. | 25.0 | 0.10 M (KNO3) | 9.11 | 15.15 | 16.92 | 28 |
| 7. | 25.0 | 0*.*10 M (KCl) | 9.09 | 15.11 | 16.81 | 14, 29 |
| 8. | 25.0 | 0.10-0.15M (KCl) | 9.11 | 15.16 | 16.88 | 15  |

 The species distribution diagram for proton-ligand equilibria of histidine is shown in Figure 3.12b. The fully protonated form XH32+ exists only below a pH of 4.0. This species loses the carboxylate proton and forms XH2+ which is a dominating species between 2 to 6 pH and represents nearly 99% of the ligand at 3.90 pH. Further increase in pH causes neutralization of the side chain proton resulting in the zwitter ionic species XH. The formation of the XH reaches a maximum of 93% of the total ligand around 7.5 pH. Above a pH of 9.0, the major species is the most anionic form of the ligand (X-) and represents the total ligand concentration around ~11.0 pH.

* **Proton-ligand equilibria of** L**-Aspartic acid:**

 Aspartic acid (2-Aminobutanedioic acid) is an acidic amino acid with two –COOH groups, one on alpha carbon atom and the other on the side chain. It is a nonessential amino acid generally found in proteins and acts as an excitatory neurotransmitter in the central nervous system and for hormone production and release. Biochemically, it plays an important role in the citric acid cycle.

 

**Figure 13**: L- Aspartic acid (XH2)

 In aqueous solution depending on the pH, L-aspartic acid may exist (Fig 14) in cationic (XH3+), neutral zwitter ionic (XH2) or anionic (XH- and X2-) forms.

|  |  |  |  |
| --- | --- | --- | --- |
| **XH3+** | **XH2** | **XH-** | **X2-** |

**Figure 14:** Successive protonated species of L-Aspartic acid

 At and above neutral pH the overall molecule is negatively charged. Due to the presence of negatively charged carboxyl group, it is found almost at the surface of proteins. The charged group can form ionic bond with various metal ions as well as dipole interaction with water which is important concept of solubility of amino acid in water.

The pH-metric titration curves of free acid (curve 1) and acid + ligand (curve 2) is shown in Figure 15a.

|  |  |
| --- | --- |
|  |  |
|  a. | 1. Titration curve of free mineral acid |  b. Species distribution diagram |
|  | 2. Titration curve for acid + Aspartic acid |  |
| [ASP] = 0.0100 mol dm-3 |  [HCl] = 0.0300 mol dm-3  | [NaOH]= 0.1773 moldm-3 |
| Temp.: 30.0 ±0. 10C  | Total volume= 50.0 cm3  | *I* = 0.1 mol dm-3 NaCl |
| Figure 15: PROTON-LIGAND SYSTEM OF L-ASPARTIC ACID |

The titration curve of the ligand possesses three buffer regions corresponding to the titration of the three protons associated with the cationic (XH3+) form of the ligand. The ligand curve is above that of the free acid in the lower pH region indicating proton association to the ligand. The experimental data were subjected to analysis by the Miniquad-75 program 13 and the best-fit chemical model obtained along with statistical parameters is shown in Table 6.

The obtained values are in close agreement with the IUPAC recommended values, with due considerations of the differences in experimental conditions. The formation constants obtained represent the equilibria.

**Table 6:** Best fit chemical model for acid-base equilibria of L-aspartic acid in aqueous medium. Temp. = 30.0 ± 0.1°C and ionic strength, *I* = 0.1 mol dm–3 (NaCl)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Species*Mlh* | *Log β0lh (SD)* | Literature14 recommended values.At 25.0 0C | Number of experimental points analysed | Sum of the squares of residuals, U  | χ2 |
| 011 |  09.58 (2) | 9.62 | 104 | 4.28 X 10-9 | 6.37 |
| 012 |  13.30 (2) | 13.32 |
| 013 |  15.16 (2) | 15.26 |



**Figure 16**: Proton-Ligand equilibria of L-ASPARTIC ACID

The formation constant *β011* is due to the protonation of the amino group of the most anionic form of the ligand. *β012* is due to the protonation of both amino and carboxylate group in the side chain of the molecule. *β013* corresponds to the overall formation constants for the complete protonation of the most anionic form of the ligand.

The species distribution diagram indicating the percentage of formation of each species against pH is shown in Figure 15 b. The fully protonated form XH3+ exists up to a pH of ~4.0. With increase in pH, it loses the ‘α- carboxylic’ proton leading to the formation of g neutral zwitter ion. The maximum extent of formation of XH2 form is 80.6% at 2.83 pH. This species exists up to ~6.0 pH. The neutral form of the ligand, with increase in pH, loses carboxylic proton of the side chain forming anionic XH- form of aspartic acid. Nearly 99% of the ligand is in this form between 5.8 to 7.8 pH. Further increase in pH leads to the formation of the most anionic X2- form by the loss of the proton associated to amino group.

* **REFERENCES:**
1. N.Vandana Jugran, Ashok Kumar Sharma, N. Jeetendra Singh, Veerma Ram, “Biological activities of hydrazide derivatives in the new millennium”, *International Journal of Pharmaceutical Chemistry*, 2 (4) (2012) pp 100-109.

# R.Narang, B.Narasimhan and B. Sharma, “A review on biological activities and chemical synthesis of hydrazide derivatives”, *Curr. Med. Chem*., 19(4) (2012) pp 569-612.

# B.K. Kaymakçioğlu, E. E. Oruç-Emre , U. Seda and A. Dimoglo “Synthesis and characterization of novel hydrazide-hydrazones and the study of their structure-antituberculosis activity”,  *European Journal of Medicinal Chemistry,* 41(11)(2006) pp 1253-1261.

1. G. Verma, A. Marella, Md. Shaquiquzzaman, M. Akhtar, Md. Rahmat Ali and Md. Mumtaz Alam, “A review exploring biological activities of hydrazones”, *J Pharm Bioallied Sci.,* 6(2) (2014) pp 69-80.
2. S. Rollas and Ş. Güniz Küçükgüzel, “Review- Biological activities of hydrazone derivatives”, *Molecules,* 12(2007) pp 1910-1939.
3. H. M. Irving and H. S. Rossotti, “Methods for Computing Successive Stability Constants from Experimental Formation Curves”, *J. Chem. Soc*. (1953) pp 3397–3405.
4. H. M. Irving and H. S. Rossotti “The Calculation of Formation Curves of Metal Complexes from pH-Titration Curves in Mixed Solvents”, *J. Chem. Soc*. (1954) pp 2904–2910.
5. G. Gran, Analyst*,* 77 (1952) p 661.
6. G. Gran, Acta *Chem. Scand.,* 4 (1950) p 559.
7. A.Ravindra Babu, J. S. V. M. Lingeswara Rao, D. Murali Krishna, and R. Sambasiva Rao, *Analytica Chimica Acta,* 306(2-3) (1995) pp 297-300.
8. G.V. Afanas’eva, T. I. Bychkova, V. G. Shtyrlin, A. R. Shakirova, and A. V. Zakharov,*Russian Journal of General Ch*emistry. 76(5) (2006) pp757-765.
9. G. Arena, E. Rizzarelli, S. Sammartano and C. Rigano. *Talanta.*, 26 (1979) p1
10. P. Gans, A. Sabatini and A. Vacca., *Inorg. Chim. Acta*., 18 (1976) p237.
11. A.E. Martell and R.M. Smith, "Critical stability constants Volumes 1 to 5", Plenum Press, NY and London, (1974-1982): Volume 1: Amino acids (1974), Volume 2: Amines (1975), Volume 3: Other Ligands (1977), Volume 4: Inorganic Complexes (1976), Volume 5: First Supplement, (1982) and Volume 6: Second Supplement (1989).
12. L.D. Pettit, “Critical survey of formation constants of Histidine Phenylalanine, Tyrosine, L-Dopa and Tryptophan”, IUPAC, *Pure & App/. Chem.,* 56(2) (1984) pp 247-292.
13. T. Kiss, I. Sovago and A. Gergely, “Critical survey of stability constants of complexes of Glycine”, IUPAC, *Pure & App/. Chem.,* 63(4) (1993) pp 597-638.
14. I. Sovago, T. Kiss and A. Gergely, “Critical survey of the stability constants of complexes of aliphatic amino acids”, IUPAC, *Pure & App/. Chem.,* 65(5) (1993) pp 1029-1080.
15. G. Berthon, “The stability constants of metal complexes of amino acids with polar side chains”, IUPAC, *Pure & App/. Chem.,* 67(7) (1995) pp 1117-1240.
16. O. Yamauchi and A. Odani, “Stability constants of metal complexes of amino acids with charged side chains- Part-I: Positively charged side chains”, IUPAC*, Pure* & *Appl. Chem.,*68(2) (1996) pp 469-496.
17. R.F. Criscent, G.F.Laniak and R.L. Erikson, “Propagation of uncertainty through geochemical code calculations”, *Geochimica et Cosmochimica Acta*, 60(1996) pp 3551-3568.
18. C.Ekberg and I. Lunden Burro, “Uncertainty analysis for some actinides under groundwater conditions”, *Journal of Statistical Computation and Simulation,* 57(1997) pp 271-284.
19. F.H. Denison and J. Granier-LaPace, “The effect of database parameter uncertainty on uranium (IV) equilibrium calculations”, *Geochimica et Cosmochimica Acta*, 69(2005) pp 2183-2191.
20. Reda A. Ammar, Nawal A. Alarfaj and Maha F. El-Tohamy, “Potentiometric Study of 1, 2-Diphenylethylenediamine Palladium (II) Complex with Some Selected Amino Acids”, *Int. J. Electrochem. Sci.,* 7 (2012) pp 1512 – 1521.
21. Y. Altun *and*  F. Koseoglu, “Stability of Copper(II), Nickel(II) and Zinc(II) Binary and Ternary Complexes of Histidine, Histamine and Glycine in Aqueous Solution”, *Journal of Solution Chemistry,* 34 (2) (2005) pp.213-231.
22. M. J. Bojczuk, P. Kaczmarek, W. Bal and K. S. Kasprzak, “Determination of the stability constants and oxidation susceptibility of nickel(II) complexes with 2′-deoxyguanosine 5′-triphosphate and l-histidine”, *J. Inorg. Biochem*. 99 (2005) p 737-746.
23. Piotr Kaczmarek, Wojciech Szczepanik and Małgorzata Jeżowska-Bojczuk, *Dalton Trans.*, (2005) pp 3653-3657.
24. Leslie D. Pettit and John L. M. Swash, ***Dalton Trans.***, (1976) 588-594.
25. H. C. Freeman and R. P. Martin, *The Journal of Biological Chemistry*, 244 (1969) p 4823.
26. B. L. Mickel and A. C. Andrews, *J*. *Am. Chem. Soc.* 77 (1955) p 1955.