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Dielectric Constant and its Influence on Protonation Equilibria of L-Serine and L-Tryptophan in Acetonitrile-Water Mixtures

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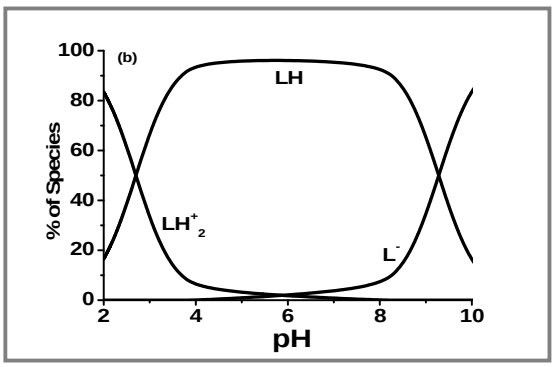
ABSTRACT

Solute-solvent interactions of L-serine and L-Tryptophan have been studied in 0–50% v/v acetonitrile-water media using pH-metric method. The protonation constants have been calculated with the computer program MINIQUAD75. Selection of the best fit chemical model of the protonation equilibria is based on standard deviation in protonation constants and residual analysis using crystallographic R-factor and sum of squares of residuals in all mass balance equations. Linear variation of protonation constants with inverse of dielectric constants of the solvent mixture has been attributed to the dominance of the electrostatic forces. Distribution of species, protonation equilibria and effect of influential parameters on the protonation constants has also been presented.

Graphical Abstract



Species distribution diagrams of L-Serine.

Keywords: Dielectric Constant, MINIQUAD75, Acetonitrile, L-serine and L-Tryptophan.

INTRODUCTION

Serine (symbol **Ser** or **S**) is an α-amino acid that is used in the biosynthesis of proteins [**1, 2**]. L-Serine possesses two protonation constants as an α-amino group (which is in the protonated $-NH_3^+$ form under biological conditions), a carboxyl group (which is in the deprotonated –COO− form in physiological conditions), and a side chain consisting of a hydroxymethyl group (see hydroxyl), classifying it as a polar amino acid. It can be synthesized in the human body under normal physiological circumstances, making it a nonessential amino acid.

Tryptophan (symbol Trp or W) is α-amino acid that is used in the biosynthesis of proteins [**1**]. Tryptophan contains an α-amino group, an α-carboxylic acid group, and a side chain indole, making it a non-polar aromatic amino acid. It is essential in humans, meaning the body cannot synthesize it: it must be obtained from the diet. In 2002, the U.S. Institute of Medicine set a Recommended Dietary Allowance (RDA) of 5 mg kg^{-1} body weight/day of Tryptophan for adults 19 years and over [**3**]

Acetonitrile is a weak base [**4**] and a much weaker acid [**5**] than water. It is a photophobic dipolar aprotic solvent and it does not form any hydrogen bond with solute species. The photophobic character of acetonitrile may arise from the possible formation of dimers which are shown to exist from IR studies. Very few studies have been reported in the literature [**6, 7**] hence the authors are reporting the protonation constants of Serine and Tryptophan in acetonitrile-water mixture.

MATERIALS AND METHODS

Experimental: 0.05 mol L^{-1} solution of L-Serine and L-Tryptophan (Avra, India) were prepared in deionized triple-distilled water by maintaining 0.05 mol L^{-1} concentration of hydrochloric acid to increase the solubility. 0.2 mol L-1 stock solution was prepared from Hydrochloric acid (Merck, India). Acetonitrile (Merck, India) were used as received. 2 mol L^{-1} Sodium chloride (Merck, India) was prepared to maintain the ionic strength in the titrand. Sodium hydroxide (Merck, India) of 0.4 mol L^{-1} and Hydrochloric acid (Merck, India) of 0.2 mol L^{-1} were prepared. All the solutions were standardized by standard methods. Thus, the concentration of the alkali was determined by titrating it with the standard oxalic acid and potassium hydrogen phthalate solutions, while the normality of hydrochloric acid was determined using standardized sodium hydroxide and the primary standard borax solutions. The strengths of alkali and mineral acid were determined using the Gran plot method [**8, 9**]. To assess the errors that might have crept into the determination of the concentrations, the data were subjected to analysis of variance of one way classification [**10**].

Alkalimetric Titrations: The pH measurements proton-ligand system were carried out in the aqueous media containing varying compositions of organic solvent 0-50% v/v (Acetonitrile) in the pH range of 2.0-10.0 maintaining an ionic strength of 0.1 with sodium chloride at 303.0± 0.1K using a digital pH meter Zeal-Tech (readability 0.01) with mechanical stirring carried by a teflon stirrer. The glass electrode was equilibrated in a well stirred acetonitrile -water mixtures containing alkali to check the complete equilibration of the glass electrode. The calomel electrode was refilled with acetonitrile-water mixtures periodically. Potassium hydrogen phthalate (0.05M) and borax (0.01M) solutions were used to calibrate the pH meter. In each titration, the titrant consisted of approximately 2.5 mmol of hydrochloric acid in a total volume of 50 mL. The amount of L-Serine and L-Tryptophan in the titrant ranged between 0.25 and 0.50 mmols. The glass electrode was equilibrated in a well stirred organic solvent–water mixture containing inert electrolyte for several days. At regular intervals, the strong acid was titrated against alkali to check the complete equilibration of the glass electrode. The details of the experimental procedure and titration assembly used in our laboratory have been given elsewhere [**11**].

Modeling Strategy: The computer program SCPHD [**12**] was used to calculate the correction factor applied to pH meter dial reading to calculate approximate protonation constants of L-Serine and L-

Tryptophan. The best fit chemical model for each system investigated was arrived at using non-linear least–squares method in the initial refinement and reliable convergence of Marquardt algorithm [**13**]. The variation of stepwise constants was analyzed mainly on electrostatic grounds on the basis of solute-solute and solute-solvent interactions.

RESULTS AND DISCUSSION

The results of best fit models that contain the type of species and overall protonation constants of L-Serine and L-Tryptophan in AN mixtures along with some important statistical parameters are given in table 1. A low standard deviation in log β indicates the precision of these parameters. The small values of U_{corr} (the sum of the squares of deviations in concentrations of ligand and hydrogen ion at all experimental points) corrected for degrees of freedom, indicate that the experimental data can be represented by the model. Small values of mean, standard deviation and mean deviation for the system confirm that the residuals are around a zero mean with little dispersion.

For an ideal normal distribution, the values of kurtosis and skewness should be three and zero, respectively. The kurtosis values in the present study indicate that the residuals form leptokurtic patterns. The values of skewness recorded in the table are between -1.95 and 4.40. These data evince that the residuals form part of a normal distribution; hence, least squares method can be applied to the present data. The sufficiency of the model is further evident from the low crystallographic R values. Thus, the statistical parameters show that best fit models represent the acid base equilibria of L-Serine and L-Tryptophan in acetonitrile–water mixtures.

Table 1. Best fit model of protonation equilibria of L-Serine and L-Tryptophan in 0-50% Acetonitrile- water mixtures

%v/v Acetonitrile	Dielectric constant	log β1 (SD)	log β2 (SD)	NP	U_{corr}	Skewness	Kurtosis	x^2	R-factor	pH range
L-Serine										
0.0	78.3	2.14 (04)	10.59 (05)	93	33.8	4.40	28.59	98.30	0.03	1.9-10.5
10.0	74.7	2.18 (05)	10.93 (22)	43	71.9	-0.43	3.76	16.14	0.08	2.8-10.0
20.0	70.5	2.02 (05)	10.61(06)	134	52.1	2.39	12.05	63.34	0.03	1.7-11.5
30.0	65.8	2.51 (04)	11.55 (08)	62	46.6	2.39	8.95	58.19	0.06	2.6-11.5
40.0	60.2	2.54 (05)	11.38 (06)	70	38.6	1.74	7.76	37.14	0.04	2.3-11.5
50.0	55.7	2.61 (04)	11.53 (07)	63	47.0	1.39	5.71	21.30	0.06	2.6-11.5
L-Tryptophan										
0.0	78.3	2.17 (08)	11.05 (09)	129	86.6	-2.79	21.49	310.19	0.04	1.4-10.5
10.0	74.7	2.12 (04)	11.06 (06)	80	43.5	2.02	9.56	34.40	0.04	2.1-10.5
20.0	70.5	2.35 (08)	11.27 (12)	80	130.7	-1.95	24.54	78.60	0.09	2.3-12.0
30.0	65.8	2.70 (03)	11.98 (05)	63	25.0	0.67	4.97	30.95	0.04	2.6-11.5
40.0	60.2	2.85 (06)	11.68 (09)	70	38.6	1.74	7.76	37.14	0.04	2.6-11.5
50.0	55.7	2.87 (04)	12.23 (06)	63	30.9	0.17	3.79	21.94	0.04	2.6-11.5

$$Ucorr = U/ (NP-m) X 10^8$$

NP = Number of points, m = number of protonation constants, SD = standard deviation.

Secondary Formation Functions: The stepwise protonation constants and number of equilibria can be determined from the secondary formation functions such as average number of protons bound per mole of ligand ($\bar{n}_H$). The pH values at half integral of $\bar{n}_H$ correspond to the protonation constants of the ligand and the number of half integrals in the pH range range of the study corresponds to the number of equilibria. Thus, two half integrals (0.5 and 1.5) versus pH in the case of L-Serine and L-Tryptophan conform the presence of two protonation–deprotonation equilibria. The maximum value of L-Serine and L-Tryptophan in the formation curve figure 1(a) and (b) is two, which clearly shows that L-Serine and L-Tryptophan has two bound protons per molecule in the pH range of present study. The typical distribution plots figure [2(a) and 2(b)] produced using protonation constants from the best fit table 1, show the existence of LH_2^+, LH and L^- in L-Serine and L-Tryptophan. The plots of **a**

versus pH are given in Figure [1(c) and 1(d)]. The negative values of **a** correspond to the number of moles of free acid present in the titrand and the number of associable protons. The positive values of **a** indicate the number of dissociable protons in the ligand molecules. The maximum value of **a** in figure 1(c) and (d) is one, which clearly infers that L-Serine and L-Tryptophan has one dissociable protons i.e one carboxylic group.

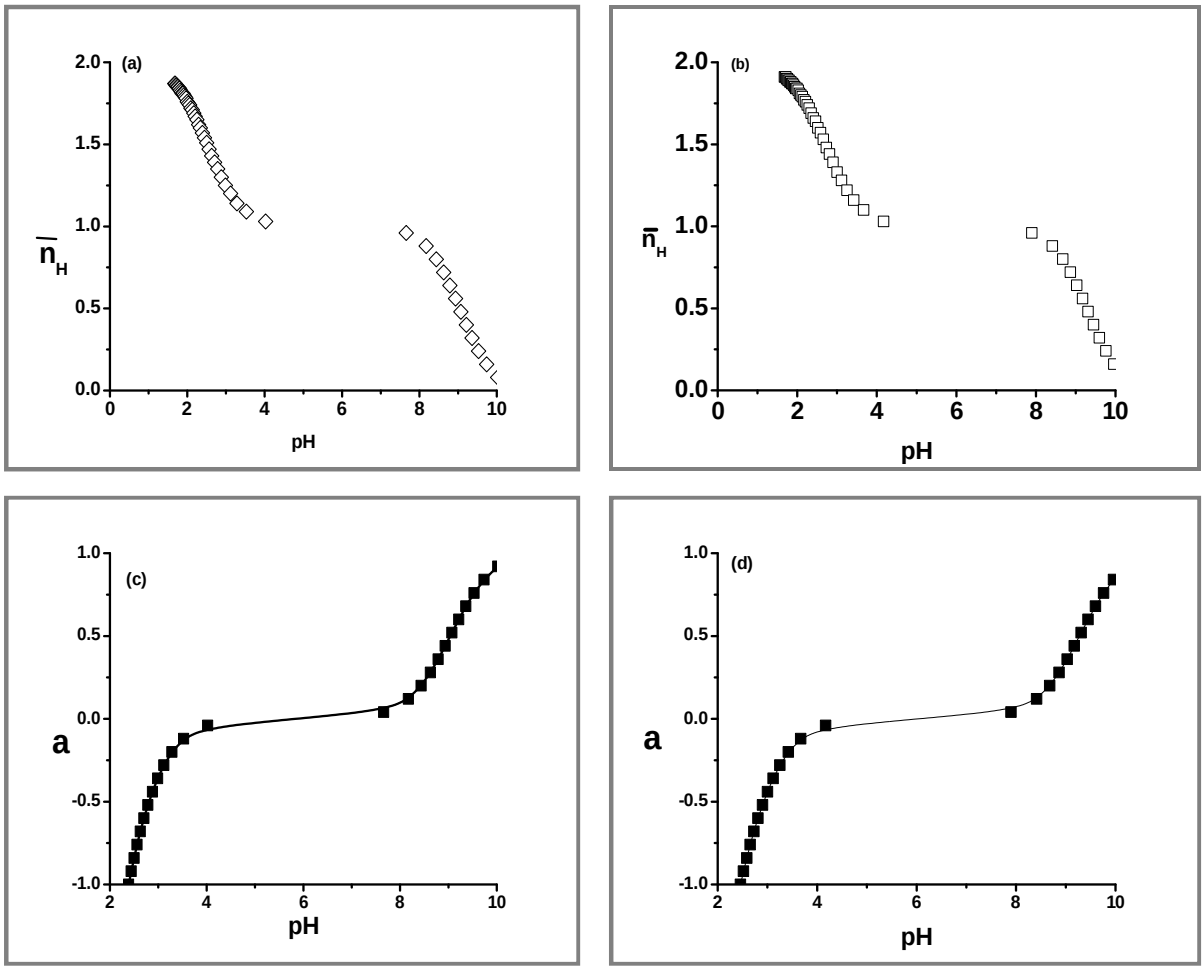


Figure 1. Plots of $\bar{n}_H$ versus pH in 30% v/v AN- water mixture: (a) L-Serine and (b) L-Tryptophan, (□) 0.25, (○) 0.375, and (Δ) 0.50 mmol, respectively. Variation of **a** with pH in 30 % v/ AN -water mixture: (C) L-Serine and (d) L-Tryptophan, respectively.

Distribution Diagrams: Typical distribution plots produced by DISPLOT [**15**] using protonation constants from the best fit models are shown in figure 2. Representative plots show the existence of, LH_2^+ LH and L^- in the case of both L-Serine and L-Tryptophan in different pH ranges. LH form of L-Serine is present to an extent of 95 % in the pH range 2.0- 10.0 and LH form of L-Tryptophan is present to an extent of 93 % in the range 2.0-10.0. The higher protonated species (in LH_2^+ the case of L-Serine and L-Tryptophan) exist below a pH of 5.0. LH_2^+ is deprotonated with increasing pH to form LH and L^- in the pH ranges 2.0-10.0 and 7.0-10.0 in the case of L- Serine and 2.0-10.0 and 8.0-10.0 in the case of Tryptophan. The corresponding protonation equilibria of both L-Serine and L-Tryptophan are shown in below.

$$LH_2^+ \rightleftharpoons LH + H^+$$

$$LH \rightleftharpoons L^- + H^+$$

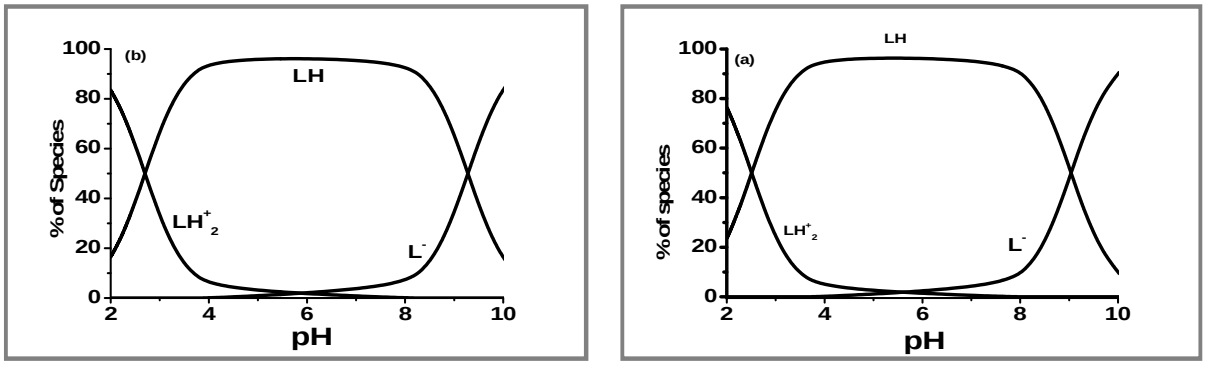


Figure 2. Species distribution diagrams of (a) L-Serine and (b) L-Tryptophan species in 30.0% v/v AN-water mixture.

Effect of Solvent: The variation of protonation constants or change in free energy with the organic solvent content depends upon two factors, electrostatic one, which can be estimated by Borns equation and non electrostatic one, which includes specific solute solvent interactions. When the electrostatic effects dominate the equilibrium proceeds, according to Borns equation [**14**], the energy of electrostatic interaction is related inversely to dielectric constant Hence, the logarithm of stepwise protonation constants (log K) should vary linearly as a function of the reciprocal of the dielectric constant of the medium. This linear increase can be attributed to ion association reaction, solute solvent interactions and solvent basicity (acidity) effects (Figure 3).

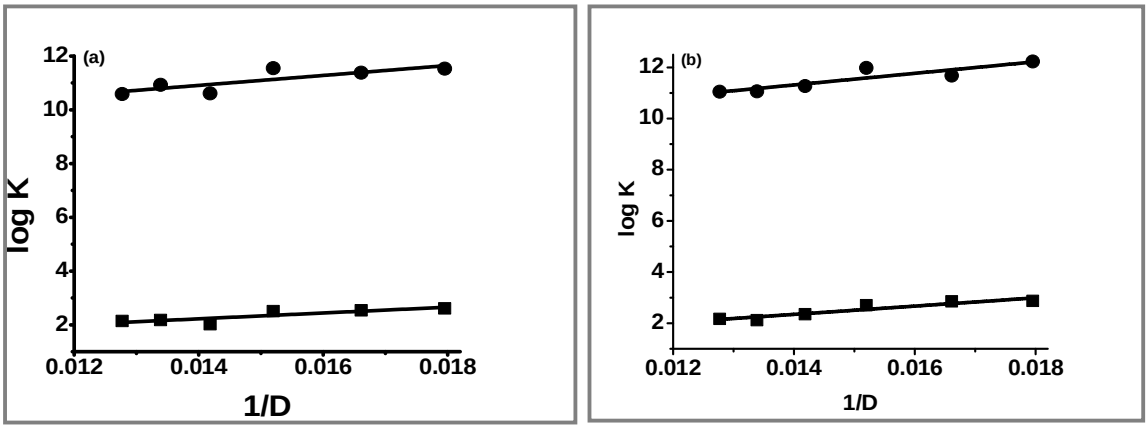


Figure 3. Variation of stepwise protonation constants (log K) of L-Serine and L-Tryptophan with reciprocal of dielectric constant (1/D) in Acetonitrile respectively: (■) log K_1, (●) log K_2.

Effect of systematic errors on best fit model: Any variation in the corrections of ingredients like alkali, mineral acid and the ligand affects the magnitude of protonation constants. Such parameters are termed influential parameters. MINIQUAD75 does not have provision to study the effect of systematic errors in the influential parameters on the magnitude of protonation constant. In order to rely upon the best fit chemical model for critical evaluation and application under varied experimental conditions with different accuracies of data acquisition, an investigation was made by introducing pessimistic errors in the concentrations of mineral acid, alkali, and ligand. The results of a typical system given in table 2, emphasize that the errors in the concentrations of alkali and mineral acid affect the protonation constants with increased errors in the concentrations of the ingredients corroborate the appropriateness of the experimental conditions. Statistically the best chemical models that represent acid–base equilibria under study should have very low standard deviation in their protonation constant (log β) values that indicate the precision of the parameters. The increased standard deviation in protonation constants and even rejection of some species on introduction of errors confirms the correctness of the proposed models. This type of investigation is significant as the data acquisition was done under varied experimental conditions with different accuracies.

Table 2. Effect of errors in influential parameters on protonation constants of L-Serine and L-Tryptophan in 30%v/v Acetonitrile-water mixtures.

Ingredient	% of error	L-Serine in Acetonitrile		L-Tryptophan in Acetonitrile	
		log K_1(SD)	log K_2(SD)	log K_1(SD)	log K_2(SD)
Alkali	0	2.14 (04)	10.59 (05)	2.17 (08)	11.05 (09)
	-5	2.81(01)	12.28(02)	2.99(02)	12.72(04)
	-2	2.63(02)	11.84(05)	2.81(02)	12.27(04)
	2	2.38(06)	11.25(11)	3.15(10)	12.29(16)
	5	2.16(10)	10.77(20)	2.40(09)	11.21(14)
Acid	0	2.14 (04)	10.59 (05)	2.17 (08)	11.05 (09)
	-5	2.09(08)	10.75(20)	2.33(07)	11.22(13)
	-2	2.36(05)	11.25(11)	2.56(04)	11.69(07)
	2	2.64(03)	11.83(05)	2.83(02)	12.26(04)
	5	2.85(02)	12.24(03)	3.04(02)	12.68(04)
Ligand	0	2.14 (04)	10.59 (05)	2.17 (08)	11.05 (09)
	-5	2.54(05)	11.51(09)	2.73(04)	11.94(06)
	-2	2.52(04)	11.54(08)	2.71(03)	11.97(05)
	2	2.49(04)	11.57(07)	2.68(03)	11.99(05)
	5	2.47(03)	11.59(06)	2.66(02)	12.01(04)

APPLICATION

The studies carried out on these systems under the present experimental conditions are useful to understand the role played by the active site cavities in biological molecules. Hence, the dielectric constant and its influence on protonation equilibria of L-Serine and L-Tryptophan in Acetonitrile-water mixtures have been carried out.

CONCLUSION

1. L-Serine and L-Tryptophan has one dissociable proton and one amino group which can associate with a proton LH_2^+, LH and L^-successively with increase in pH.

2. The log values of protonation constants of L-Serine and L-Tryptophan increase linearly with decreasing dielectric constant of acetonitrile-water mixtures. This indicates the dominance of electrostatic forces in the protonation-deprotonation equilibria.

3. The effect of systematic errors in the influential parameters on the protonation constants shows that the errors in the concentrations of alkali and mineral acid affect the protonation constants more than those in the concentration of ligand solutions.

REFERENCES

[1]. Nomenclature and Symbolism for Amino Acids and Peptides. IUPAC-IUB Joint Commission on Biochemical Nomenclature, **1983**.
[2]. Nomenclature and symbolism for amino acids and peptides (IUPAC-IUB Recommendations 1983), *Pure Appl. Chem.*, **1984**, 56(5), 595–624, ,
[3]. Institute of Medicine, Protein and Amino Acids. Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids, Washington, DC: The National Academies Press, **2002**, 589–768.
[4]. A. W. Loubengayer, D. S. Sears, Donor-acceptor bonding III methyl cyanide addition compounds of boron trichloride and boron trifluoride, *J. Am. Chem. Soc.*, **1945**, 67, 164-167.
[5]. J. S. Fritz, Differential titration of amines, *Anal. Chem.*, **1953**, 25, 407-411.
[6]. Ch. Nageswara Rao, M. Ramanaiah, B. B. V. Sailaja, Evaluation of Protonation Constants of L-Asparagine and Maleic Acid in Aqueous and Aqueous Solutions of Ethylene Glycol, *Int. J. Sci. Res.*, **2014**, 3, 23-26.

[7]. Ch. Nageswara Rao, V. Gowri Kumari, B. B. V. Sailaja, Solvent effect of protonation eqilibria of L-Asparagine and Maleic Acid in dimethyl sulfoxide, *J. Indian Chem. Soc.,* **2014**, 91, 1021-1027.
[8]. G. Gran, Analyst, Determination of the equivalence point in potentiometric titrations, **1952**, 77, 661-671.
[9]. G. Gran, Equivalence volumes in potentiometric titrations, *Anal Chim Acta*., **1988**, 206, 111-123.
[10]. R. S. Rao, G. N. Rao, Computer Applications in Chemistry, *Himalaya Publishing House: Mumbai*, **2005**, p302.
[11]. CH. Nageswara Rao, M. Ramanaiah, B.B.V. Sailaja, *Chem. Spec. Bioavail.,* Influence of dielectric constant on protonation equilibria of maleic acid and L-asparagine in acetonitrile water–mixtures, **2014**, 26, 266-271.
[12]. G. Gans, A .Sabatini, A. Vacca, An improved computer program for the computation of formation constants from potentiometric data, *Inorg. Chim. Acta*, **1976**, 18, 237-239.
[13]. Ch, Nageswara Rao, B. Srinu, V. Gowri Kumari, B. B. V Sailaja. Computer-augmented modeling studies of Pb(II) and Cd(II) complexes with maleic acid in ethylene glycol–water mixture, *Chem. Spec. Bioavailab*., **2016**, 28, 66-71.
[14]. M. Born, Volumen und hydratationswarme der ionen. *Z. Phys.*, **1920**, 1, 45-48.