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Effect of Non-Ionic Micelles on Protonation Equilibria of L-Leucine and Isoleucine

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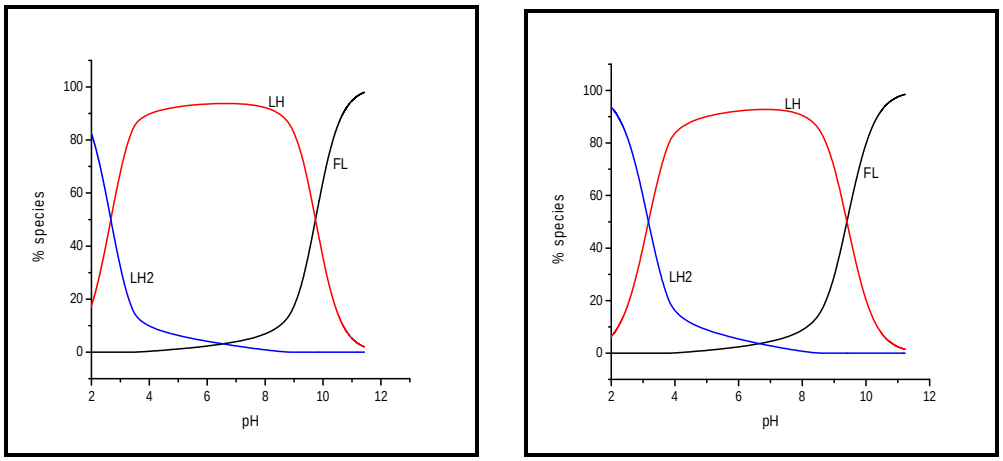
ABSTRACT

Protonation equilibria of L-Leucine and Isoleucine have been studied in varying concentrations (0.0-2.5% v/v) of Triton X-100 (TX100) solution maintaining an ionic strength of 0.16 mol dm^{-3} at 303K using pH metric method. The protonation constants have been calculated with the computer program MINIQUAD 75 and the best fit models are arrived at based on statistical grounds employing crystallographic R factor, skewness, χ2 and kurtosis. These protonation constants values have been found to shift in micellar media as compared to those in pure water. The differences in the values have been attributed to the solvent properties of the interfacial and bulk phases involving contribution from the micellar surface potential in the case of charged micelles. The trend of log values of step-wise protonation constants with mole fraction of the medium have been explained based on electrostatic and non-electrostatic forces operating on the protonation equilibria. Distributions of species, protonation equilibria and effect of influential parameters on the protonation constants have also been presented.

Graphical Abstract



Species distribution diagrams of (A) L-Leucine, (B) Isoleucine in 1.0% v/v TX100-water mixture.

Keywords: Protonation constants, L-Leucine, Isoleucine, Triton X-100, MINIQUAD 75.

INTRODUCTION

Triton X-100 (poly ethylene glycol P- (1, 1, 3, 3-tetramethyl butyl) –phenyl ether (or) octyl phenol ethoxylate (or) polyoxy ethylene octylphenyl ether) is a non-ionic surfactant which has a hydrophobic polyethylene oxide group and a hydrophobic group. The hydrocarbon group is a 4-(1, 1, 3, 3-tetramethylbutyl) – phenyl group. It can be used to permeabilize eukaryotic cell membranes. It is also used in conjunction with zwitterionic detergents to solubilize membrane proteins in their native state. They can solubilise, concentrate and compartmentalize ions and molecules [**1-3**]. The effect of non-ionic micelles on protonation equilibria of L-Leucine and Isoleucine in TX100-water mixtures has been investigated. An insight into the protonation equilibria is also helpful in understanding the metal-ligand equilibria associated with these ligands.

Leucine [**4**] (abbreviated as Leu or L) (*2-Amino-4-methylpentanoic acid*) is an α-amino acid with the chemical formula $HOOCCH(NH_2)CH_2CH(CH_3)_2$. It is used in the biosynthesis of proteins. It contains an α-amino group (which is in the protonated $-NH_3^+$ form under biological conditions), an α-carboxylic acid group (which is in the deprotonated $-COO^-$ form under biological conditions), and a side chain isobutyl group, making it a non-polar aliphatic amino acid. It is an essential amino acid, meaning the body cannot synthesize. It must be obtained from the diet. Human dietary sources are foods that contain protein, such as meats, dairy products, soy products, beans and legumes. L-leucine, like other essential amino acids, helps maintain muscle mass. For this reason, many bodybuilders and athletes take leucine supplements. Leucine [**5**] is neutral essential ketogenic amino acid and forms an acetoacetate and acetate. It is a branched chain amino acid and taken up by brain and muscle. In leucine metabolism, transamination gives χ-keto isocaproic acid, which is converted into corresponding CoA, this is similar to oxidative decarboxylation of α-ketoglutarate and pyruvate. The enzyme complex is very important in the body of living organism. A deficiency of the enzyme causes maple syrup urine disease. In this disease the urine gives odor of maple syrup or burnt sugar, deterioration is rapid and results in mental retardation. Leucine is the only dietary amino acid that has the capacity to stimulate muscle protein synthesis [**6**]. As a food additive, L-leucine has E number E641 and is classified as a flavor enhancer [**7**]. The Food and Nutrition Board (FNB) of the U.S. Institute of Medicine set Recommended Dietary Allowances (RDAs) for essential amino acids in 2002. Leucine requires for adults 19 years and above is 42 mg kg^{-1} body weight day^{-1} [**8**].

Isoleucine (abbreviated as Ile) *(*2-amino-3-methylpentanoic acid*)*, as evidenced by its name, is the isomer of leucine and higher homologue of valine. Ehrlich was the first one who discovered Isoleucine in 1904, from fibrin, a protein involved in blood-clot formation. Isoleucine is an essential, neutral, genetically coded amino acid. It is not synthesized in animals, hence it must be ingested. Isoleucine has several significant roles in biological systems [**9-16**]. Isoleucine is one of the three branched chain amino acids (BCAAs) along with leucine and valine. Relative to the other two BCAAs, isoleucine is intermediate for its ability to induce muscle protein synthesis (stronger than valine, but much weaker than leucine) but is able to significantly increase glucose uptake and the usage of glucose during exercise. Isoleucine is only a good supplement to purchase when wanting to increase glucose uptake; it is outperformed by leucine for inducing muscle protein synthesis. Recent studies have shown that Ile may be useful in the treatment of metabolic syndrome, diabetes, adiposity, and hepatic encephalopathy [**17-19**]. Furthermore, Ile derivatives have been targeted for the development of drugs, such as (2S, 3R, 4S)-4-hydroxyisoleucine [**20**], neuropeptide glutamic acid-isoleucine [**21**] and N-methyl-4-isoleucine cyclosporine [**22**]. Traditionally, lle has been extracted from animal tissues and produced through chemical synthesis. Both Isoleuine and leucine stimulates the brain in order to produce mental alertness.

MATERIALS AND METHODS

All the chemicals used in this investigation were of analytical reagent grade purity. Solutions of 0.05M L-Leucine (Merck, India),0.05M Isoleucine (Merck, India), 0.2M Hydrochloric acid (Merck,

India), 0.4M of sodium hydroxide (Merck, India) and Triton-x (Merck, India) were prepared. AR sample of Triton X-100 (TX100, Merck) is used as such and its purity was checked by determining critical micellar concentration (CMC) conductometrically. The CMC value of TX100 is 0.0089 M, at 303 K. Sodium chloride (Merck, India) of 2.0 M was prepared to maintain the ionic strength in the titrand. Triple-distilled deionized water was used for preparation of all the solutions. The acid and base solutions were standardized by standard methods. The concentration of the alkali was determined by titrating it with standard oxalic acid and potassium hydrogen phthalate solutions, while the normality of hydrochloric acid was determined using the standardized sodium hydroxide and the primary standard borax solutions. To assess the errors that might have crept into the concentrations, the data were subjected to analysis of variance of one way classification (ANOVA) using the computer program COSWT. The strengths of alkali and mineral acid were determined using the Gran plot method [**23-24**].

The titrimetric data were obtained by using calibrated ELICO (Model LI-120) pH-meter (readability 0.01). The glass electrode was equilibrated in a well stirred solvent solution containing inert electrolyte. The effects of variations in asymmetry, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of glass electrode were accounted for in the form of correction factor [**25**]. For the determination of protonation constants of Leucine and Isoleucine, initially titrations of strong acid with alkali were carried out at regular intervals to check whether complete equilibration was achieved. Then the calomel electrode was refilled with solvent solution of equivalent composition as that of the titrand. The titrations were carried out in media containing varying amounts of surfactants maintaining an ionic strength of 0.16 mol dm^{-3} with NaCl at 303K. In these titrations, the titrand consisted of mineral acid and ligand, in the presence and absence of metal ion, in a total volume of 50 cm^3. Titrations were performed by adding each time 0.1cm^3 portions of sodium hydroxide (0.4 mol dm^{-3}) to the titrand. The pH meter reading was recorded only after a constant value was displayed. Typical duplicate titrations showed that equilibration is fast and titration data do not differ by more than 0.02 units.

Alkalimetric titrations: Alkalimetric titrations were carried out in media containing varying compositions of TX100-water (0.5–2.5% v/v) maintaining an ionic strength of 0.16 $molL^{-1}$ with sodium chloride at 303 ± 0.05 K. An Elico LI-120 pH meter was used. Potassium hydrogen phthalate (0.05 mol L^{-1}) and borax (0.01$molL^{-1}$) solutions were used to calibrate the pH meter. In each titration, the titrand consisted of approximately 1mmol of hydrochloric acid. The amounts of the ligands in the titrands ranged between 0.25 and 0.50mmols. The glass electrode was equilibrated in a well stirred TX100-water mixture containing inert electrolyte for several days. At regular intervals strong acid was titrated against alkali to check the complete equilibration of the glass electrode. The calomel electrode was refilled with TX100-water mixture of equivalent composition as that of the titrand. The details of experimental procedure and titration assembly have been detailed elsewhere [**26**]. Typical alkalimetric titration curves are given in figure 1.

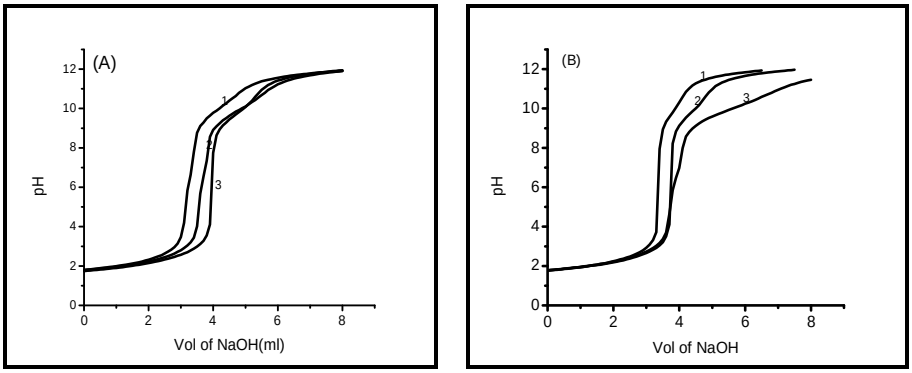


Figure 1. Alkalimetric titration curves in 0.5% v/v TX100-water mixtures: **(A)** L-Leucine **(B)** Isoleucine; (a) 0.25, (b) 0.375 and (c) 0.50 mmol, respectively.

Modeling strategy: The approximate protonation constants of L-Leucine and Isoleucine were calculated with the computer program SCPHD [27]. The best fit chemical model for each system investigated was arrived at using MINIQUAD75 [28]. The variation of stepwise protonation constants was analyzed on electrostatic grounds on the basis of solute-solute and solute-solvent interactions.

RESULTS AND DISCUSSION

Residual Analysis: In data analysis with least squares methods, the residuals (the differences between the experimental data and the data simulated based on the model parameters) are assumed to follow Gaussian or normal distribution [29-32]. When the data are fit into the models, the residuals should be ideally equal to zero. Further, a model is considered adequate only if the residuals do not show any trend. Respecting the hypothesis of the least squares analysis, the residuals are tested for normal distribution. Such tests are $\chi 2$, skewness, kurtosis and R-factor. These statistical parameters of the present data shows that the best fit models portray the acid-base equilibria of L-Leucine and Isoleucine in TX100-water mixtures, as discussed below.

$\chi 2$ test: $\chi 2$ is a special case of gamma distribution whose probability density function is an asymmetrical function. This distribution measures the probability of residuals forming a part of standard normal distribution with zero mean and unit standard deviation. If the $\chi 2$ calculated is less than the table value, the model is accepted.

Crystallographic R-test: Hamilton's R factor ratio test is applied in complex equilibria to decide whether inclusion of more species in the model is necessary or not. In pH-metric method the readability of pH meter is taken as the R limit' which represents the upper boundary of R beyond which the model bears no significance. When there are different numbers of species the models whose values are greater than R-table are rejected. The low crystallographic R-values given in table 1 indicate the sufficiency of the model.

Table 1. Best fit chemical model of protonation equilibria of L-Leucine and Isoleucine in TX-100 water mixtures. Temp= 303 K, Ionic strength= 0.16 mol dm^{-1}.

% v/v Triton-x	$\log\beta_{mlh}$ (SD)		NP	U_{corr}	Skewness	χ^2	R-Factor	Kurtosis	pH-Range
	011	012							
Leucine									
0.0	9.72(6)	12.09(8)	95	76.5	-4.93	56.23	0.0491	39.23	1.8-9.9
0.5	9.45(4)	12.33(7)	104	54.7	0.16	15.15	0.0398	3.90	2.0-9.9
1.0	9.62(5)	12.78(9)	100	75.1	0.69	6.48	0.0490	2.89	2.1-9.9
1.5	9.84(5)	13.02(8)	91	52.4	1.06	6.13	0.0414	4.01	2.0-9.9
2.0	9.60(1)	12.03(1)	80	2.25	0.08	12.40	0.0093	4.92	2.0-10.0
2.5	9.51(1)	11.99(1)	85	3.48	0.30	4.68	0.0118	3.01	2.0-9.9
Isoleucine									
0.0	9.58(2)	11.93(3)	102	15.11	-2.44	23.53	0.0200	15.15	2.0-10.0
0.5	9.44(4)	12.22(7)	109	58.5	0.06	12.46	0.0409	3.13	2.0-10.0
1.0	9.40(4)	12.57(9)	104	73.03	0.78	8.77	0.0477	4.77	2.0-10.0
1.5	9.78(4)	12.89(7)	103	45.9	1.08	8.99	0.0374	3.97	2.0-10.0
2.0	9.65(1)	12.15(2)	83	6.59	0.09	2.87	0.0159	2.71	2.0-10.0
2.5	9.52(1)	12.00(1)	85	2.79	0.12	10.14	0.0105	2.83	2.0-10.0

Skewness: It is a dimensionless quantity indicating the shape of the error distribution profile. A value of zero for skewness indicates that the underlying distribution is symmetrical. If the skewness is greater than zero, the peak of the error distribution curve is to the left of the mean and the peak is to the right of the mean if skewness is less than zero. The values of skewness recorded in table 2 are between -4.93 and 1.08. These data evince that the residuals form a part of normal distribution; hence, least-squares method can be applied to the present data.

Kurtosis: It is a measure of the peakedness of the error distribution near a model value. For an ideal normal distribution kurtosis value should be three (mesokurtic). If the calculated kurtosis is less than three, the peak of the error distribution curve is flat (platykurtic) and if the kurtosis is greater than three, the distribution shall have sharp peak (leptokurtic). The kurtosis values in the present study indicate that the residuals form nearer mesokurtic and leptokurtic pattern in the case of both L-Leucine and Isoleucine.

Table 2. Effect of systematic errors in influential parameters on the protonation constants of L-Leucine and Isoleucine in Triton-x-water mixtures in 1.5 v/v

log β mlh (SD)					
Ingredient	% Error	Leucine		Isoleucine	
		011	012	0 11	0 12
Alkali	-5	10.32(11)	13.86(15)	10.23(7)	13.69(12)
	+5	9.43(4)	12.35(6)	9.35(4)	12.20(6)
	-2	10.02(6)	13.33(10)	9.95(5)	13.194(8)
	+2	9.68(4)	12.75(7)	9.61(3)	12.61(6)
Acid	-5	9.47(3)	12.27(5)	9.39(3)	12.12(4)
	+5	10.26(12)	13.92(18)	10.16(8)	13.76(15)
	-2	9.69(4)	12.72(6)	9.63(3)	12.57(5)
	+2	9.99(7)	13.35(11)	9.92(3)	13.21(9)
Ligand	-5	9.80(5)	13.06(9)	9.73(4)	12.92(8)
	+5	9.87(4)	13.00(6)	9.82(3)	12.86(6)
	-2	9.82(5)	13.04(8)	9.76(4)	12.9(7)
	+2	9.86(4)	13.01(6)	9.79(4)	12.88(6)

Effect of systematic errors on best fit model: Any variation in the concentrations of ingredients like alkali, mineral acid and ligand affects the magnitudes of protonation constants. Such parameters are called influential or dangerous parameters. 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 influential parameters. 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 more than that of the ligand.

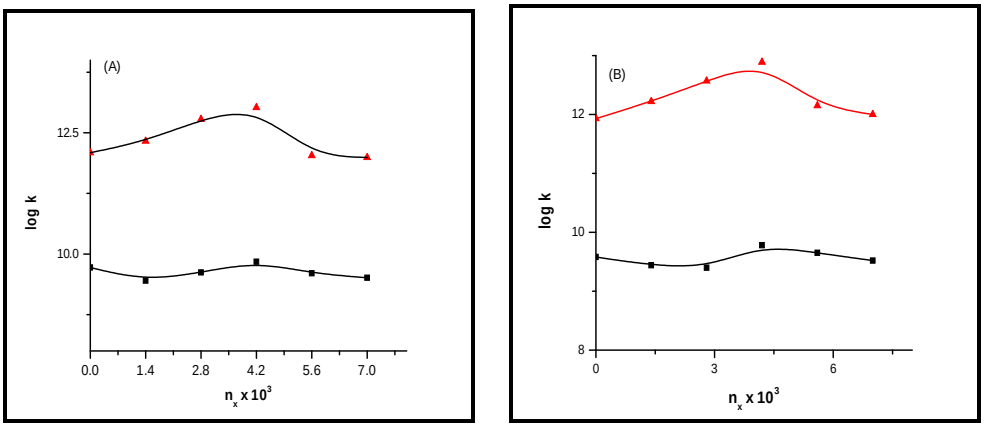


Figure 2. Variation of stepwise protonation constant (log K) with mole fraction of solvent. (A) L-Leu and (B) Isoleu in TX100-water mixtures ; (■) log K1, (▲) log K2.

Effect of solvent: Effect of solvent on protonation constant depends upon electrostatic and non-electrostatic factors. Born's classical treatment holds good in accounting for the electrostatic contribution [33] which is related to dielectric constant. Hence, the logarithm of step-wise protonation constant (log K) should vary linearly as a function of the reciprocal of dielectric constant (1/D) of the

medium. The log K values in present study are linearly increasing (Figure 2) with decreasing dielectric constant of the medium in both the Ligands.

Distribution diagrams: The distribution plots (Figure 3) produced using the protonation constants from the best fit models (Table 1) show the existence of LH^{2+}, LH and L^- in the case of both L-leucine and Isoleucine in different pH ranges. The LH^{2+} species is predominant at low pH. The pH increases with the decrease of concentration exponentially. The LH species exists at pH 3-11 and L^- species exists at pH 9 -13.

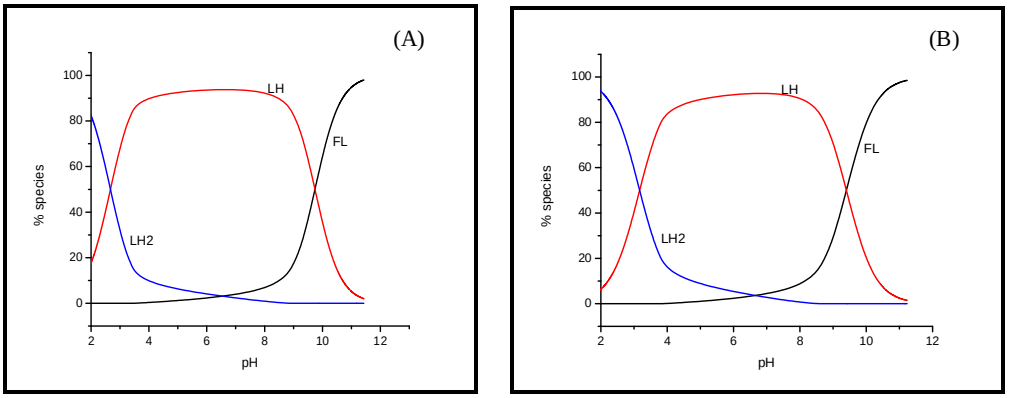


Figure 3. Species distribution diagrams of (A) L-Leucine, (B) Isoleucine in 1.0% v/v TX100-water mixture.

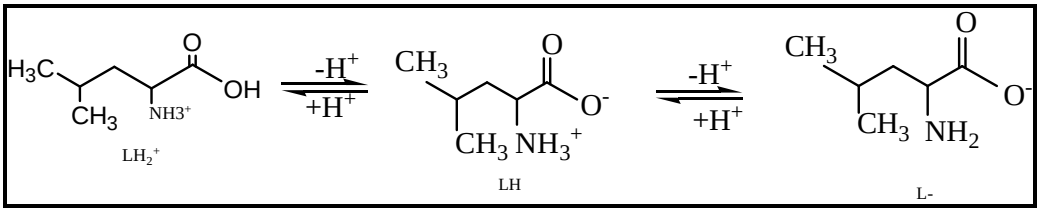


Figure 4. protonation-Deprotonation equilibria of L-Leucine.

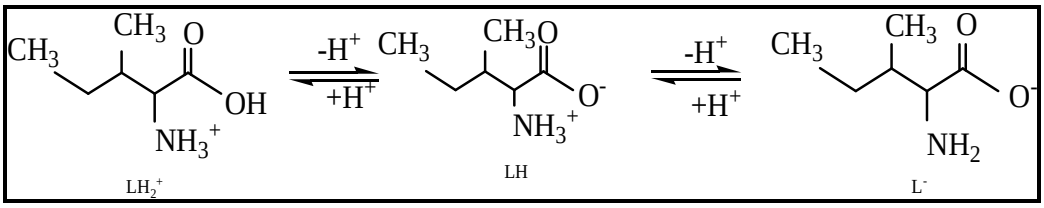


Figure 5. Protonation-Deprotonation equilibria of Isoleucine.

APPLICATION

Speciation determines the behavior of trace elements in a system, and in the human organism speciation has a great effect on bioavailability, distribution and toxicity. 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 speciation studies on the protonation equilibria of L-Leucine and Isoleucine in varying compositions of Triton-x-water mixtures have been carried out.

CONCLUSIONS

1. Both L-Leu and Ile exists as LH_2^+ at low pH and gets deprotonated with the formation of LH and L^- successively with increase in pH.
2. The log values of protonation constants of L-Leucine and Isoleucine increased almost linearly with increasing mole fraction of TX100-water mixtures indicating the dominance of electrostatic forces in the protonation-deprotonation equilibria and hydrogen bonding in TX100.
3. The effect of systematic errors in the influential parameters shows that the errors in the concentrations of alkali and mineral acids will affect the protonation constants more than that of the ligand.

REFERENCES

[1]. E. Pelizetti, E. Pramaro, Analytical applications of organized molecular assemblies *Anal. Chim. Acta.*, **1985**, 169, 1-29.
[2]. Anita Sarin, Rama Pande, Protonation behavior of hydroxamic acids, *J. Applicable Chem.*, **2014**, 3(6), 2633-2637.
[3]. A. D Mudawadkar, G. H. Sonawane, T. J Patil, Thermodynamics of Micellization of Nonionic Surfactant Triton X-100 in Presence of Additive Poly-N-Vinyl- Pyrrolidone using Clouding Phenomenon, *Orient. J. Chem*, **2013,** 29(1), 227-233.
[4]. B. S. Garg, Dwived Poonam, Investigations and chemical speciation of ternary complexes of bivalent metal ions with cephalexine as primary ligand and glycine and valine as secondary ligands, *J. Indian Chem. Soc.*, **2006,** 83(3), 229.
[5]. Schonheimer R, The dynamic state of body constituent, Harvard Univ. Pr. Cambridge (Mass) **1942**.
[6]. M. R. Etzel, "Manufacture and use of dairy protein fractions". *The Journal of Nutrition*, **2004**, 134 (4): 996S–1002S.
[7]. Winter, Ruth, A consumer's dictionary of food additives (7th ed.). New York: Three Rivers Press, **2009**, ISBN 0307408922.
[8]. Institute of Medicine , "Protein and Amino Acids". Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington, DC: **2002,** the National Academies Press. pp. 589–768.
[9]. S. J. O' Meara, B. T Kinsella, Investigation of the effect of the farnesyl protein transferase inhibitor R115777 on isoprenylation and intracellular signalling by the prostacyclin receptor, *Br J. Pharmacol.*, **2004**, 143, 318- 330.
[10]. Zhu, K.; Ding, X.; Julotok, M.; Wilkinson, B. *J. Appl. Environ. Microbiol*. **2005**, 71 (2), 8002-8007.
[11]. G. Guan, T. Todo, M. Tanaka, G. Young, Y, Nagahama, Isoleucine-15 of rainbow trout carbonyl reductase-like 20β-hydroxysteroid dehydrogenase is critical for coenzyme (NADPH) binding, *PNAS*, **2000**, 97 (7), 3079-3083.
[12]. L. B. Wang, J. G Shen, S. Z. Zhang, K. F. Ding, S. Zheng, *World J. Gastroenterol.*, **2004**, 10 (9), 1297-1300.
[13]. Y. Takasata, S. Momma, Q. R Smith, Kinetic analysis of cerebrovascular isoleucine transport from saline and plasma, *J. Neurochem.*, **1985**, 45 (4), 1013- 1020.
[14]. M. Kawabe, M. Sano, A. Hagiwara, S. Tamao, S. Suzuki Shirai, Studies on Biologically Significant Copper (II) / Manganese (II) / Uranyl (II) – Isoleucine Binary Complexes, T. *Food Chem. Toxicol.*, **2006**, 44(2), 278-285.
[15]. P. Schadewaldt, A. Bodner-Leidecker, H. W. Hammen, U Wendel, Significance of L-Alloisoleucine in plasma for diagnosis of Maple Syrup Urine Disease, *Clinical Chem.*, **1999**, 45, 1734-1740.
[16]. H. Xu, Y.Zhang, X. Guo, G, Zhao, Isoleucine Biosynthesis in Leptospira interrogans Serotype lai Strain 56601 Proceeds via a Threonine-Independent Pathway, *J. Bacteriol.*, **2004**, 186(16), 5400-5409.

[17]. L. K. Bak, P. Iversen, M. Sorensen, S. Keiding, H. Vilstrup, Meta-bolic fate of isoleucine in a rat model of hepatic encephalopathy and in cultured neural cells exposed to ammonia. *Metab Brain Dis.*, **2009**; 24(1), 135-45.
[18]. M. Doi, I. Yamaoka, M. Nakayama, K. Sugahara, F. Yoshizawa, Hypoglycemic effect of isoleucine involves increased muscle glucose uptake and wholebody glucose oxidation and decreased hepatic gluconeogenesis. *Am J Physiol-Endocrinol Metab*, **2007**, 292, 683-93.
[19]. J. Nishimura, T. Masaki, M. Arakawa, M. Seike, H. Yoshimatsu, Iso-leucine prevents the accumulation of tissue triglycerides and up-regulates the expression of PPARalpha and uncoupling protein in diet-induced obese mice, *J. Nutr.*, **2010**, 140(3), 496-500.
[20]. J. Ogawa, T. Kodera, S.V. Smirnov, M. Hibi, N. N. Samsonova, A novel L-isoleucine metabolism in Bacillus thuringiensis generating (2S,3R,4S)-4-hydroxyisoleucine, a potential insulinotropic and anti-obesity amino acid, *Appl Microbiol Biotechnol.*, **2011,** 89(6), 1929-38.
[21]. A. M. Attademo, T. A. Rondini, B. C. Rodrigues, J. C. Bittencourt, M. E. Celis, Neuropeptide glutamic acid-isoleucine may induce luteinizing hormone secretion via multiple pathways, *Neuroendocrinology*, **2006**. 83(5-6), 313-24.
[22]. H. Wang, Y. Zhang, T. Wang, H. You, J. Jia, N-methyl-4-isoleucine cyclosporine attenuates CCl induced liver fibrosis in rats by interacting with cyclophilin B and D, *J Gastroentero Hepatol*, **2011**, 26(3), 558-67.
[23]. G. Gran, Determination of the equivalence point in potentiometric titrations, *Part II Analyst*, **1952**, 77, 661.
[24]. G. Gran, Equivalence volumes in potentiometric titrations, *Anal. Chim. Acta.*, **1988**, 206,111-123.
[25]. K. V. Santhee Devi, B. Rama Raju, G. N. Rao, Effect of dielectric constant on protonation equlibria of L-dopa and 1, 10 -phenanthroline in dioxan-water mixtures, *Acta Chim. Slov.*, **2010**, 57, 398.
[26]. B. Rama Raju, K. V. Santhee Devi, G. N. Rao, Formation of binary complexes of Co(II), Ni(II) and Cu(II) with L-Dopa in Dioxan-water mixtures, *Bull. Chem. Soc. Ethiop.*, **2011**, 25, 43-52.
[27]. G. N. Rao, Complex equilibria of some biologically important metal ions in aquo-organic media, PhD thesis, Andhra University, India, **1989**.
[28]. P. 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.
[29]. R. S. Rao G. N. Rao, Computer applications in chemistry. Himalaya Publishing House, Mumbai, **2005**, pp 277-351.
[30]. K. V. Santhee Devi, B. Rama Raju, G. N. Rao, pH-metric Investigation on Mixed-Ligand Complexes of Ca(II), Mg(II) and Zn(II) with L-Dopa and 1, 10- Phenanthroline in Propylene glycol -Water Mixtures, Research. Review: *J. Chem.*, **2012**, 1, 13-22.
[31]. M. Ramanaiah, S. Gouthama sri, B. B. V. Sailaja, Chemical speciation of PbII, Cd-II and Hg-II binary complexes of L-phenylalanine in CTAB-water mixtures, *J. Indian Chem. Soc.*, **2014**, 91, 351-357.
[32]. 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. Resea.*, **2014**, 3, 23-26.
[33]. M. Born, A Perspective on "volume and heat of hydration of ions", *J. Phys.*, **1920**, 1, 45.