Impact of C-TAB on Protonation Equilibria of L-Leucine and Isoleucine

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ABSTRACT *In this study, the protonation constants of L-Leucine and Isoleucine have been measured in various concentrations (0.0-2.5% w/v) of CTAB at 303 K at an ionic strength of 0.16 mol L-1 using a pH metric method. They were calculated using the computer program MINIQUAD75 which are found to be distinct in micellar medium than in pure water. The change of log values of protonation constants with mole fraction of the medium have been illustrated on the basis of interactions between solute and solvent. In addition, distributions of species and alteration of influential parameters on the protonation constants were also presented in this study.*

Keywords: *Protonation equilibria, MINIQUAD75, Cetyltrimethylammoniumbromide, L-Leucine, Isoleucine*

INTRODUCTION

Cetyltrimethylammonium bromide (CTAB) is a cationic surfactant which affects the properties of physiological systems and can solubilize, concentrate, compartmentalize ions and molecules [1] and can shift acid–base equilibria. Protonation constants of various amino acids have been reported in cationic micellar medium [2-4]. Protonation constants of L-Leucine and Isoleucine were determined in other micellar media [5]. The present study is an attempt to examine the effect of cationic micelle on the protonation constants of L-Leucine and Isoleucine.

Leucine [6] (represented as **Leu** or **L**) (*2-Amino-4-methylpentanoic acid*) and Isoleucine (abbreviated as **Ile**) (*2-amino-3-methylpentanoic acid*) are essential, non-polar, aliphatic amino acids which play a vital role in biological systems [7-14]. Both the amino acids contain two dissociable protons, one in the carboxylic group and the other in the amino group. Leucine produces an enzyme complex which is much needed in the body of living organism whose deficiency causes maple syrup urine disease which results in mental retardation. Recent studies have shown that Ile is useful in the treatment of metabolic syndrome, diabetes, adiposity, and hepatic encephalopathy [15-17]. Also, research has shown that Ile derivatives have been used for the development of drugs, such as (2S, 3R, 4S) -4 – hydroxyisoleucine [18], neuropeptide glutamic acid-isoleucine [19], andN-methyl-4-isoleucine cyclosporine [20].

CHEMICALS AND PROCEDURE

Chemicals

L-Leucine and Isoleucine solutions of 0.05 mol L-1 were prepared in triple distilled water by maintaining 0.05 mol dm-3 acid (HCl) concentration to increase the solubility. Cetyltrimethylammonium bromide (CTAB) of analytical reagent grade obtained from Merck was used as received. To maintain the ionic strength in the titrant, Sodium chloride (Merck) of 2 mol L-1 was prepared. Solutions of 0.4 mol L-1 Sodium hydroxide and 0.2 mol L-1 HCl were also prepared and were standardized by standard methods.

Alkalimetric titrations: The titrations with alkali were performed in media having different compositions of CTAB-water (0.5–2.5% w/v) keeping an ionic strength of 0.16 mol L-1 with sodium chloride at 303.00 ± 0.05 K using an Elico LI-120 pH meter. The pH meter is calibrated with Potassium hydrogen phthalate (0.05 mol L-1) and borax (0.01 mol L-1) solutions. The glass electrode was equilibrated in a CTAB-water mixture containing inert electrolyte for several days. At regular intervals, an acid – base titration was carried out to check for complete equilibration. In each titration, the titrant consists of 1 mmol of hydrochloric acid and 0.25 to 0.50 mmols of the ligand and it is titrated by adding 0.1cm^3 of sodium hydroxide each time to the titrant. The curves for the alkalimetric titrations are given in Figure 1.

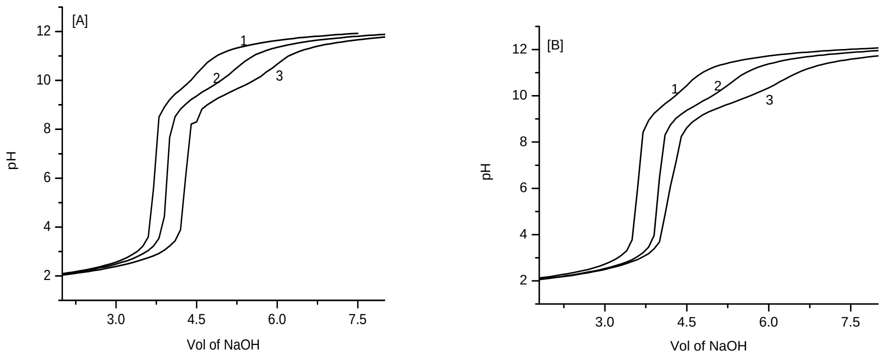

Fig. 1: Alkalimetric titration curves in 1.5% w/v CTAB-water mixtures: **(A)** L-Leucine **(B)** Isoleucine; (a) 0.25, (b) 0.375 and (c) 0.50 mmol, respectively.

Modeling strategy: The computer program SCPHD [21] was used to apply the correction factor to the pH meter reading. The protonation constants of L-Leucine and Isoleucine in different concentrations of CTAB - water mixture were calculated using MINIQUAD75 [22]. The variation of stepwise protonation constants was examined on the basis of interactions between solute-solute and solute-solvent. The best fit model having the type of species and protonation constants together with certain statistical parameters are given in table 1.

Table 1. Best fit chemical model of protonation equilibria of L-Leucine and Isoleucine in CTAB water mixtures. Temp= 303 K, Ionic strength= 0.16 mol dm-3.

% v/v ctab	$\log\beta_{mlh}$ (SD) 11	12	NP	U_{corr}	Skew-ness	χ^2	R-Factor	kurtosis	pH-Range
				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	9.65(3)	12.04(4)	96	27.3	-0.95	5.25	0.0354	4.92	2.0-10.5
1.0	9.68(1)	12.41(2)	90	7.52	0.06	26.31	0.0174	6.18	2.05-10.0
1.5	9.49(2)	12.18(3)	106	17.01	-0.32	20.75	0.027	5.24	2.0-10.5
2.0	10.33(6)	13.61(11)	99	77.42	-0.23	100.26	0.0533	5.82	2.0-10.5
2.5	10.37(6)	13.71(11)	94	71.19	-0.12	43.4	0.0526	4.84	2.05-10.5
				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	9.59(2)	12.27(4)	101	21.01	-2.37	26.2	0.0306	16.37	2.05-10.5
1.0	9.71(3)	12.81(5)	116	31.75	-0.27	5.66	0.0344	5.52	2.0-10.5
1.5	9.49(1)	12.31(2)	102	9.57	0.11	22.75	0.0189	5.10	2.0-10.0
2.0	10.4(7)	13.93(12)	104	75.98	0.13	6.85	0.051	3.54	2.05-10.5
2.5	10.37(6)	13.92(11)	106	60.48	0.12	4.15	0.044	3.24	2.05-10.5

Results and Discussion

The values of low standard deviation (SD) in log β and U_{corr} (sum of the squares of deviations in concentrations of ligand and hydrogen ion at all experimental data points corrected for degree of freedom) show that the experimental data can be depicted by the model. The values of kurtosis in table 1 signify that the residuals form nearer mesokurtic and leptokurtic patterns. The skewness values (-0.12 and 0.12) explain that the residuals form a part of normal distribution and therefore, least squares method is applicable to the present data. The acceptability of the model is more apparent from the low crystallographic R-values. These statistical parameters show that the best fit model describes the acido-basic equilibria of L-Leucine and Isoleucine in cationic micellar medium.

Effect of systematic errors on best fit model: Alteration in the concentrations of components like alkali, mineral acid and ligand affects the magnitudes of protonation constants. Such parameters are called influential parameters. As MINIQUAD75 cannot be used to study the effect of systematic errors in the influential parameters, the change in protonation constants with the variation in different components was examined by introducing errors in the influential parameters and the results are featured in Table 2.

Table 2: Effect of systematic errors in influential parameters on the protonation constants of L-Leucine Isoleucine in 1.0% w/v ctab-water mixtures

		log βmlh (SD)			
Ingredient	% Error	Leu		Isoleu	
		11	12	11	12
Alkali	-5	10.12(5)	13.2(7)	10.18(5)	13.69(10)
	+5	9.28(1)	11.73(1)	9.27(2)	12.08(4)
	-2	9.85(2)	12.71(4)	9.89(3)	13.14(7)
	+2	9.52(1)	12.13(1)	9.53(2)	12.5(4)
Acid	-5	9.32(1)	11.67(1)	9.32(1)	12.00(2)
	+5	10.06(5)	13.26(7)	10.1(5)	13.77(13)
	-2	9.54(1)	12.1(1)	9.55(2)	12.47(4)
	+2	9.83(2)	12.72(4)	9.86(3)	13.16(7)
Ligand	-5	9.64(1)	12.43(2)	9.65(3)	12.84(6)
	+5	9.72(1)	12.39(2)	9.76(2)	12.78(5)
	-2	9.67(1)	12.42(2)	9.68(3)	12.82(5)
	+2	9.7(1)	12.4(2)	9.73(2)	12.79(5)

Effect of micelles: The variation of protonation constants with solvent depends upon two factors, viz., electrostatic and non-electrostatic interactions. Born's classical treatment considers for the electrostatic contribution [23]. According to this treatment, the logarithm of step-wise protonation constants (log K) should vary linearly with the mole fraction of the medium. Such linear variation of the protonation constants of L-Leucine and Isoleucine in CTAB-water mixture (Fig 2) shows the dominance of electrostatic interactions.

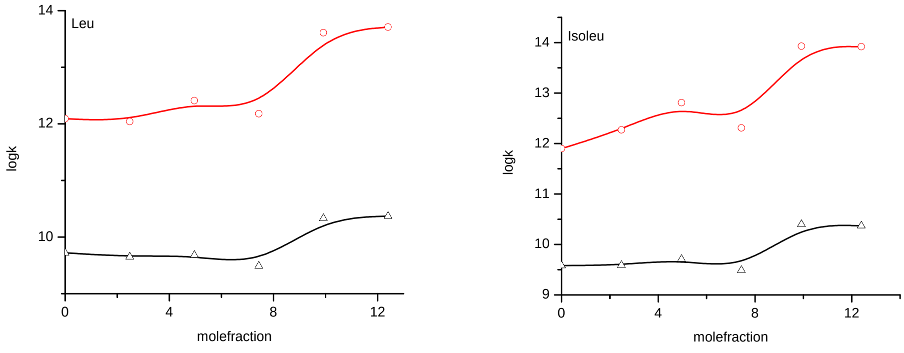

Fig. 2: Variation of stepwise protonation constant (log K) with mole fraction of solvent. (A) L-Leu and (B) Isoleu in CTAB-water mixtures; (Δ) log K1, (O) log K2

Distribution diagrams

The species distribution diagrams (Figure 3) occurred from the protonation constants show the existence of LH_2^+, LH and L^- in case of both L-leucine and Isoleucine in different pH ranges. The protonation-deprotonation equilibria of both the ligands are shown in Figure 4 and 5.

The LH_2^+ species is predominant at low pH. As the pH increases its concentration decrease exponentially. The LH species exists at pH 3-9 and L^- species exists at pH 8 -11.

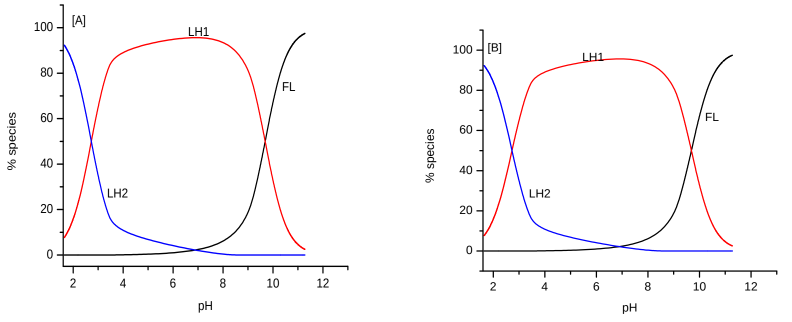

Figure 3. Species distribution diagrams of (A) L-Leucine and (B) Isoleucine in 1.5% w/v CTAB-water mixture.

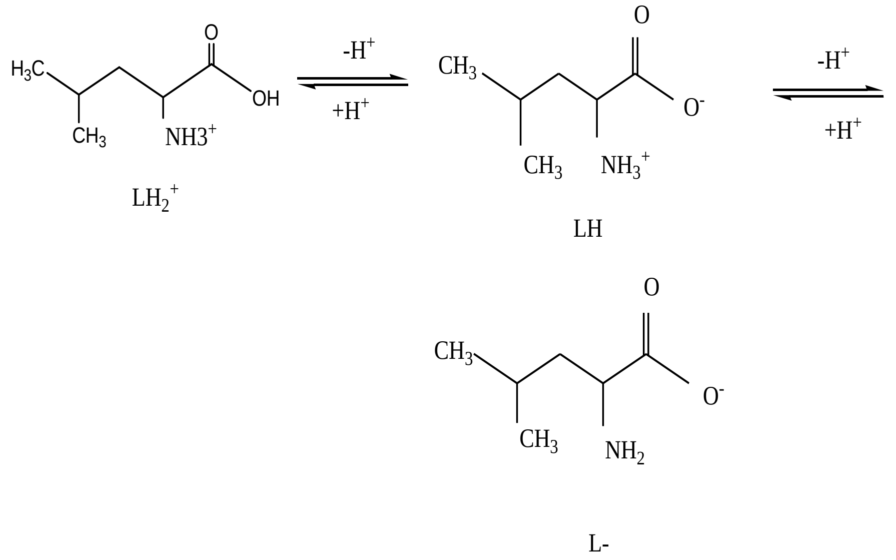

Fig 4: Protonation-Deprotonation equilibria of L.Leucine

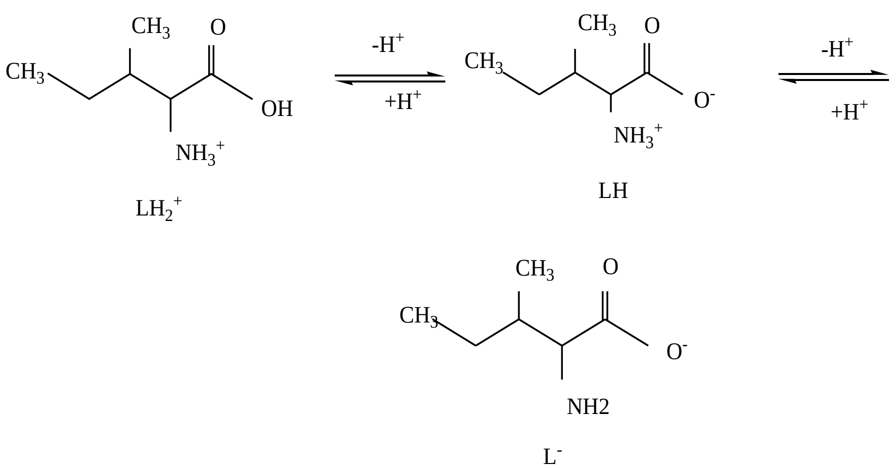

Fig 5: Protonation - Deprotonation equilibria of Isoleucine

APPLICATIONS

Chemical speciation interprets the occurrence of distinct chemical forms of metal in a sample and also has a great effect on bioavailability, distribution and toxicity in a human body. The research performed is helpful in understanding the role played by the active site cavities in biological molecules. Thus, the speciation studies have been carried out on the protonation equilibria of L-Leucine and Isoleucine in different concentrations of CTAB – water mixtures.

Conclusions

1. Both L-Leu and Ile have two dissociable protons and exists as LH_2^+ at low pH and gets deprotonated with the formation of LH and L- successively with increase in pH.
2. The change of log values of protonation constants of L-Leucine and Isoleucine linearly with increasing mole fraction of CTAB-water mixtures indicate the dominance of electrostatic forces in the protonation-deprotonation equilibria and hydrogen bonding.

3. Alteration in the concentrations of components like acid and ligand affects the protonation constants more than that of the change in alkali.

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