EFFECT OF IONIC AND NON-IONIC MICELLES ON PROTONATION EQUILIBRIA OF L-ORNITHINE: A POTENTIOMETRIC STUDY

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ABSTRACT

The protonationconstants of L-ornithine (L-Orn) in polyethylene glycol 400 (PEG-400, a neutral surfactant) and tetrabutylammonium bromide(TBAB, a cationinc surfactant) micellemedia have been determined by Calvin-Wilson titration method which is based on pH metric titration. The pH metric study was carried outat 0.5-2.5%micellar solutions (PEG-400, v/v and TBAB, w/v)at a temperature of 298 K andan ionic strength of 0.16 mol dm^{-3}sodium chloride.In the first step, pH meter correction factor and K$_w$were determinedfrom SCPHD program and their valueswere used asinputs for MINIQUAD75 program to get the protonation constants. The species distribution diagram, obtained from SIM refined dataand generated by origin85, shows the relative aubandance of LH_3^{2+}, LH_2^+, LH and L^- as a function of pH. The variation in the values of protonation constants between aqueous medium and both micellar media can beattributed to the different electronic environment and intrinsic solvent characteristics of micellar media. In case of ionic surfactants, the electrostatic micellar surface is an additional contributing factor.

Keywords

Protonation Constants, L-Ornithine, PEG-400, TBAB, Micelles;

1. Introduction

L-Ornithine (L-Orn) is a non-protein 2,5-diaminopentanoic acid whose metal complexes are insoluble inwater and soluble in most organic solvents. It is known to form stable metal complexes in a variety of solvents differing in the degree of solubility. L-Orn complexes of bio-essential metals play an important role in chemotherapeutic applications[1-2]. L-Orn also helps to build muscles, reduces body fats, removes toxic ammonia from liver and is the source of polyamines in maintaining physiological system.

Acid association constants are one of the important parameters to predict the extent of ionization of a molecule with respect to given pH. The ligand exists in different forms in different systems and properties of each form are different.This information is essential in the estimates of pharmacokinetics and pharmacodynamic properties like absorption, distribution metabolism and excretion. Knowledge of the distributions of various protonated species of the ligands against pH is also an essential requirement to understand the various interactions that exist in a solution containing a metal and several ligands.

Since the high concentrations of the metal complexes of L-Orn are insoluble in water, organic solvents are used for their determinations or extractions. Green chemistry requires the elimination of toxic organic solvents and So the author has chosen surfactants preferably since they have better ability of increasing solubility of poorly soluble compounds containing analytes. Moreover, even though surfactants are not also environmentally friendly, the amount of surfactants required in aqua-surfactant medium in the complex reactions is very small and thus, do not pose much environmental load to the ecosystem.

Surfactants form micelles above a certain concentrationsand can solublize various kinds of solutes.They have also profound effects on the metal ligand complex equilibria. Surfactants are preferred to other organic solvents, since surfactant modified procedures are more sensitive, selective and accurate compared to earlier methods[3]. The effects of micellar media non-ionic, polyethylene glycol 400 (PEG-400) and ionic, tetrabutylammonium bromide (TBAB) on acid-base and metal-ligand complex equilibria has been investigated by takingL-Ornligand. The results are reported in this paper[4-12].

2. Materials and Methodology

2.1. Materials

All the reagents used, throughout this study, were of analytical grade. All solutions were prepared in triply distilled water. Nitrogen gas was purged to expel dissolved oxygen and carbon dioxide or any other dissolved gases. A ~0.05 mol dm^{-3} L-Orn (Sigma-Aldrich, India), was prepared in 0.05 mol dm^{-3} HCl. A ~ 0.4 mol dm^{-3} of sodium hydroxide (Merck, India) was prepared and standardized against potassium hydrogen phthalate. It was regularly Gran-titrated[13,15] to ensure complete removal of carbonates. A ~0.2 mol dm^{-3} HCl (Merck, India) solution was standardized against 0.4 mol dm^{-3} NaOH standard solution. TBAB (Merck, India), PEG-400 (Merck, India) and L-Orn (Sigma-Aldrich, India) were used as received. An ionic strength of 0.16 mol dm^{-3} wasmaintainedby 2.0 mol dm^{-3} solutionof NaCl (Merck, India).

2.2. Methodology

Alkalimetric titrations were conducted in micellar media using Metrohm 877 titrino plus auto-titrator (Switzerland) (readability 0.001) having an integrated electrode (0-14 pH range) at the temperature of 298 K. The electrode was calibrated with 0.1 mol dm^{-3} potassium hydrogen phthalate (pH 4.01) and 0.05 mol dm^{-3} borax solutions (pH 9.18). It was found that the three measurements of replicate titrations differ by not more than 0.02 units.

After glass electrode calibration by freshly prepared solutions, it was equilibrated in a well stirred micelle-water solution containing inert electrolyte. Complete equilibration was checked through titration of the strong acid with alkali at regular intervals prior to alkalimetric titrations. Whenever the experiments were conducted in different percentage of surfactants, the electrodes were equilibrated in that particular surfactant percentage.

In each titration, the titrand consisted of requisite volumes of hydrochloric acid, sodium chloride and the ligand, the values of which were calculated based on achieving total fixed ionic strength of 0.16 mol dm^{-3} in a total volume of 50 mL. Alkalimetric titrations were then conducted by adding 0.05 mL of 0.4 mol dm^{-3} NaOH in combination electrode of the auto-titrator. The titrations were conducted in both the surfactants at various concentrations of the surfactants (0.5–2.5% v/v) and at different concentrations of L-Orn (0.25, 0.375 and 0.50 mmol) for the triplicate measurements.

3.Results and Discussion

3.1. Analysis of Data

Calvin-Wilson titration technique modified by Irving and Rossotti[13-15] was used for the study of protonation equilibria of the ligand (L-Orn). This technique is as follows: requisite volumes of hydrochloric acid (to give an overall concentration of ~2.0 – 5.0 × 10^{-2} mol dm^{-3}), sodium chloride (to maintain the ionic strength 0.16 mol dm^{-3}) and ligand (2.5-7.5 × 10^{-3}mol dm^{-3}in different experiments) in the absence and presence of a micellar media, in a total volume of 50.0 cm^{3} were titrated with ~0.4 mol dm^{-3} sodium hydroxide at 25.0 ± 0.1 ^{o}C. After the addition of each aliquot (0.05 cm^{3}) of sodium hydroxide, the pH meter data were automatically saved by the instrument.The alkalimetric titration curves are given in **Figure 1**.

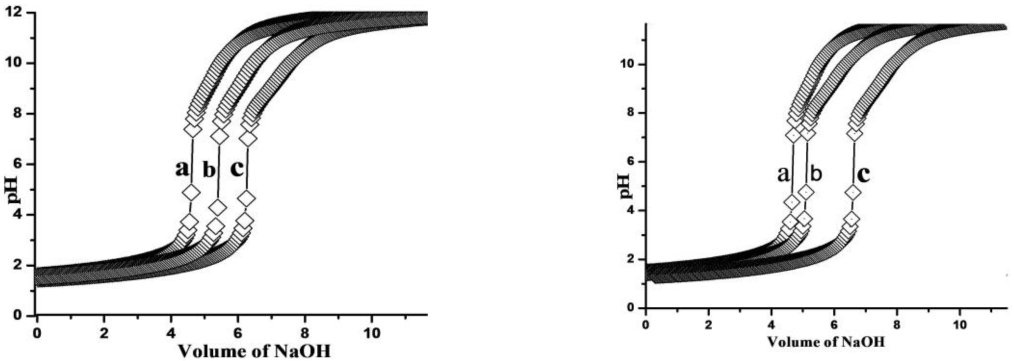


Figure 1. Alkalimetric titration curves in 1.5% (v/v) (A) TBAB -water mixtures and (B) PEG 400-water mixtures:L-ornithine; (a) 0.25, (b) 0.375 and (c) 0.50 mmol, respectively.

The effect of activity coefficient, dissolved carbon dioxide, sodium ion error, variations in the asymmetric and liquid junction potentials on the response capacity of pH electrode were considered in the form of $K_w$and correction factor obtained from SCPHD[16] program(developed in this laboratory). The protonation constants of L-Orn were determined by MINIQUAD75[17] program using correction factor and K_w as initial estimates. The best fit modelcontainsthe three protonation constants logK_1, log K_2 and log K_3 corresponding to LH^+, $LH_2^{2+}$and LH_3^{3+} shown in **Table 1**.

Table 1. Comparison of protonation constants of L-Orn in PEG-400 andTBAB with literature values

Log K_1	Log K_2	Log K_3	Solvent	Method	Reference
10.73	8.62	1.94	Aqueous	Glass electrode	[18]
10.59	8.93	2.11	Aqueous	Glass electrode	[19]
10.77	8.65	1.705	Aqueous	pH-metry	[20]
10.76	8.65	1.94	Aqueous	pH-metry	[21]
10.40	8.65	2.09	Aqueous	Potentiometry	Present study
10.39	8.60	1.92	PEG-400 (non-ionic micellar)	Potentiometry	Present study
10.06	8.27	1.66	TBAB (ionic micellar)	Potentiometry	Present study

3.2. Protonation constants

The protonation constants of L-Orn in PEG-400 and TBAB micellar media (0.0–2.5% v/v) were found to be in the pH range 1.48–10.5 and 1.6-10.73, respectively. The best fit chemical models for L^-, LH, LH_2^+ and LH_3^{2+} species along with the overall stablity constants (log β_1=log K_1, log β_2=log K_1+ log K_2 and log β_3= log K_1+ log K_2 + log K_3) and stepwise stability constants (log K_1, log K_2 and log K_3) are given in **Table 2**.

Table 2. Best fit model for acid-base equilibria of L-Orn in PEG-400 and TBAB-micellar media at 0.16 mol dm^{-3} ionicstrength and298 K.Temp

surfactant	$\log\beta_{mlh}$ (SD)			NP	U_{corr}	Skewness	χ^2	R-Factor	kurtosis	pH-Range	log K_1	log K_2	log K_3
	011	012	013	PEG-400 (% v/v)									
0.0	10.40(1)	19.05(2)	21.14(2)	120	0.6	0.16	29.69	0.00338	7.39	1.55-11.0	10.4	8.65	2.09
0.5	10.35(1)	18.87(2)	20.81(2)	92	0.4	-0.01	6.09	0.00318	3.30	1.7-10.3	10.35	8.52	1.94
1.0	10.38(5)	18.98(5)	20.82(5)	111	1.8	0.74	10.97	0.00517	8.09	1.5-10.1	10.38	8.6	1.84
1.5	10.39(1)	18.99(1)	20.91(1)	76	0.1	-0.08	6.21	0.00186	3.35	1.8-10.5	10.39	8.6	1.92
2.0	10.41(4)	18.99(4)	20.93(5)	107	2.0	0.89	17.82	0.00611	4.41	1.6-10.3	10.41	8.58	1.94
2.5	10.41(1)	19.17(3)	21.08(5)	71	1.7	0.59	11.01	0.00861	3.04	1.85-10.7	10.41	8.76	1.91
				TBAB (% w/v)									
0.0	10.40(1)	19.05(2)	21.14(2)	120	0.6	0.16	29.69	0.00338	7.39	1.55-11.0	10.4	8.65	2.09
0.5	10.26(1)	18.83(2)	20.66(2)	218	3.9	0.33	19.52	0.00887	3.06	1.67-10.75	10.26	8.57	1.83
1.0	10.24(2)	18.75(2)	20.23(2)	153	1.7	-0.28	6.10	0.00674	3.29	1.8-10.0	10.24	8.51	1.48
1.5	10.06(5)	18.33(6)	19.99(6)	109	3.8	-0.05	15.83	0.00790	4.16	1.3-9.9	10.06	8.27	1.66
2.0	9.96(3)	18.23(5)	19.70(6)	111	4.4	-0.38	12.52	0.00879	3.86	1.45-10.5	9.96	8.27	1.47
2.5	9.69(4)	17.78(7)	18.64(9)	113	7.0	-0.08	30.0	0.01117	6.91	1.45-10.65	9.69	8.09	0.86

$U_{corr} = U/(NP-m) \times 10^8$, where m= number of species; NP= number of experimental points

Distribution diagrams: The type and percentage of the major form of the complexes have a strong dependence on pH which is represented by species distribution diagrams.Origin85has been used to generate the species distribution diagrams (**Figure 2**). It can be observed from the diagram that below pH~2.0, L-Orn(L) occurs mostly in the LH_3^{2+} form and with the increase in pH, it undergoes successive deprotonation to form LH_2^+ and LHspecies. The species distribution diagram indicates that the LH_2^+ form exists upto the pH of ~8.0 with a maximum formation of 98% between the pH of 4.0 to 7.0. The ligand is in the LH form between the pH of 8.0 to 11.0. The free ligand, FL starts at pH 10.0 and exists up to pH 12.0.

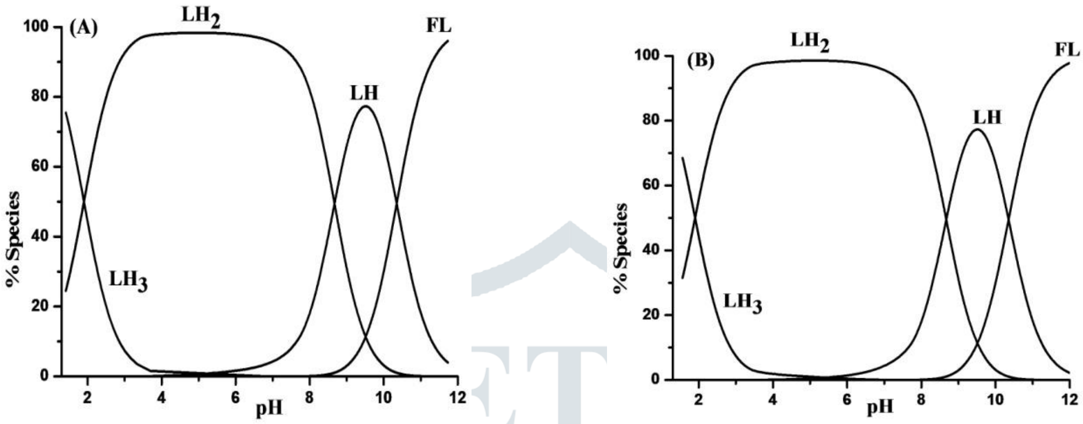


Figure 2. Species distribution diagrams of L-ornithine in (A) 1.5% w/v PEG 400-water and (B) 1.5% v/v TBAB-water mixtures.

Effect of systematic errors on best fit model:

The best fit model could represent the experimental data as standard deviation (SD) and U_{corr} (sum of squares of residuals in all mass-balance equations) for the log β values are very low. MINIQUAD or other similar programs do not have any built in provision torecognize or overcome errors.In order to investigate the expected errors, the influential parameters were subjected to deliverately introduced pessimistic errors. The results (given in **Table 3**) clearly showed that the errors due to change in concentrations of alkali and mineral acid have affected the values of the protonation constants profoundly compared to that of the ligand concentration as expected.

Table 3: Effect of errors due to concentrations of components of titration mixtures on the protonation constants of L-Orn.

		log βmlh (SD)					
Ingredient	**% Error**	**PEG-400**			**TBAB**		
		0 11	**012**	**013**	**011**	**012**	**013**
	0	10.39(1)	18.99(1)	20.91(1)	10.06(5)	18.33(6)	19.99(6)
Alkali	-5	11.47(28)	20.98(25)	23.66(26)	10.92(14)	19.89 (13)	21.79(13)
	-2	10.75(3)	19.71(3)	21.89(4)	10.35(4)	18.89(3)	20.64(3)
	+2	10.03(2)	18.30(3)	6.21(4)	9.80(6)	17.79(9)	19.35(10)
	+5	9.47(5)	17.23(10)	18.43(14)	9.42(7)	16.91(17)	18.34(18)
Acid	-5	9.44(7)	16.81(19)	17.63(23)	9.44(5)	16.81(19)	17.63(23)
	-2	9.82(3)	17.78(7)	19.17(9)	9.82(6)	17.78(9)	19.17(10)
	+2	10.32(1)	18.88(1)	20.79(1)	10.32(6)	18.88(5)	20.79(6)
	+5	10.95(6)	20.16(6)	22.79(6)	10.77(17)	19.74(15)	22.07(15)
Ligand	-5	10.34(5)	18.99(5)	20.97(5)	10.02(5)	18.34(5)	20.13(5)
	-2	10.37(5)	18.98(5)	20.88(5)	10.04(5)	18.34(5)	20.04(5)
	+2	10.40(5)	18.98(5)	20.77(5)	10.08(5)	18.33(5)	19.94(6)
	+5	10.42(5)	18.97(5)	20.69(5)	10.10(6)	18.32(5)	19.86(7)

Effect of Micelles

The dielectric constants of micellar media are lower when compared to aqueous medium. Due to anisotropic water distribution in micelles, water concentration is higher at the surfacethan at the core of the micelle which is totally hydrophobic. As a result, the microviscosity, polarity and degree of hydration are not uniform in micellar media[6,7].

In ionic surfactants,there is an ion-pair coupling between oppositely changed solute species and head group of surfactantleading to electrostatic interactions.These electrostatic interactiona are absent in non-ionic surfactants[8-10].

The effect of non-ionic surfactant (PEG-400) and anionic surfactant (TBAB) on the protonation equilibria of L-Orn have been investigated. In the case of the first protonation constant, logK_1in the presence of TBABis smaller compared to log K_1 value in aqueous and PEG-400 medium **(Table 4).** Theprotonated speciesis destabilized in the presence of TBAB, a cationic surfactant.This is due to thehigh electrostatic repulsions of protonated species by cationic site of the TBAB micelle. The acid-base equilibrium shifts in favor of dissociation of LHto L^-resulting in smaller protonation constant values. In the case of the second (log K_2) and third protonation (log K_3) constants, the species are highly charged compared to LH and the effect of the micellar media is more significant for higher charged species. The electrostatic repulsions are much more for highly charged species. Therefore the logK_2 and log K_3values of LH_2^+ and LH_3^{2+} were found to be much lower in TBAB compared to water and PEG-400.The log K_1 values are marginally lower in PEG-400 compared to water. This marginal difference is due to the absence of electrostatic factor.The dielectric constant of micellar phase is lower than that of water and is the only factor contributing to stabilization of L^- form. The association equilibrium is shifted to the left hand side and protonation constant decreases (**Figure 3**). The electrostatic repulsions increase, with the increase in percentage of TBAB, resulting in decrease in the log K_1, log K_2 and log K_3 values. With increase in percentage of PEG-400, the log K_1, log K_2 and log K_3 values are almost constant since this extra stabilization/destabilization is absent in PEG-400[23].

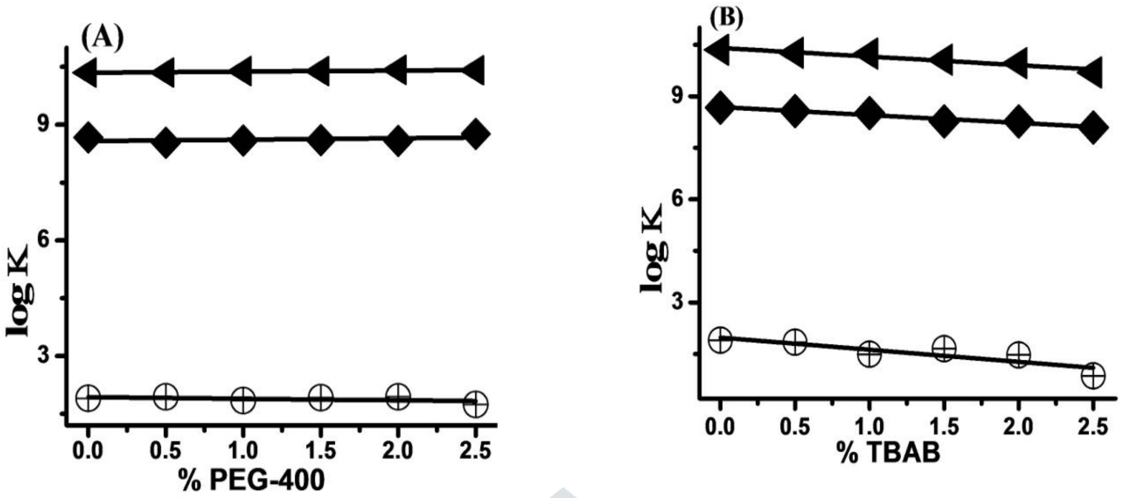
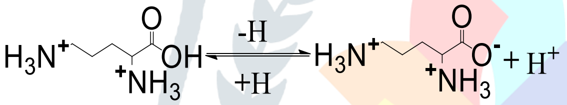
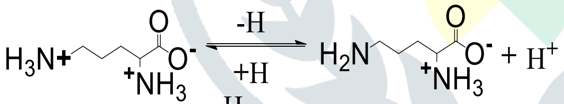
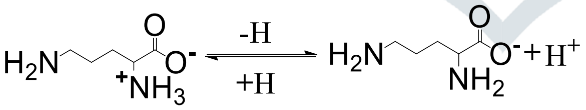


Figure 3. Variation of stepwise protonation constant (log K) of L-Orn with percentage of surfactants. (A) PEG 400-water and (B) TBAB-water mixtures; (⊗) log K_1, (◊) log K_2, (Δ) log K_3.

Table4. Protonation equilibria of L-orn and its step-wise protonation constants

S.No.	Protonation equilibria of L-orn	Step-wise protonation constants	Water	PEG-400	TBAB
1.	 Cationic $LH_3^{2+} \underset{+H}{\overset{-H}{\rightleftharpoons}} LH_2^{+} + H^{+}$ Zwitterion	Log K_1	10.40	10.39	10.06
2.	 Zwitterion $LH_2^{+} \underset{+H}{\overset{-H}{\rightleftharpoons}} LH + H^{+}$ Zwitterion	Log K_2	8.65	8.60	8.27
3.	 Zwitterion LH $\underset{+H}{\overset{-H}{\rightleftharpoons}} L^{-} + H^{+}$Anionic	Log K_3	2.09	1.92	1.66

4. Conclusions

- Theprotonation constatnts were determined in (PEG-400) aneutral and TBAB) an ionic micellar media using Calvin-Wilson techinqueand data refined by MINIQUAD75 program.
- In both the media, L-Orn exists as LH_3^{2+} at low pH (~2.0), LH_2^{+}, LH and L^{-}species were formed with consecutive deprotonationat higher pH (~8.0 -11.0).
- The values of log β of L-Orn in the TBAB micellar media are lower compared to values in aqueous medium and PEG-400.
- The low values are due to the high electrostatic repulsions of protonated species by cationic site of the TBAB micellar media.

Acknowledgments

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