



## SOLVENT EFFECT ON PROTONATION EQUILIBRIA OF L-CYSTEINE AND L-THREONINE IN AQUEOUS SOLUTIONS OF CTAB-WATER MIXTURES

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### ABSTRACT

The protonation constants of L-Cysteine and L-Threonine have been studied pH metrically in various concentrations (0.0-2.50% v/v) of CTAB- water mixtures maintaining an ionic strength of 0.16 mol L<sup>-1</sup> at 303.0 K. The protonation constants have been calculated with the computer program MINQUAD75 and the best fit chemical models are selected based on statistical parameters. Linear variation of step-wise protonation constants (log K) with reciprocal of dielectric constant of the solvent mixture has been attributed to the dominance of the electrostatic forces.

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## INTRODUCTION

Cysteine is an important source of sulfur in human metabolism, and although it is classified as a non-essential amino acid, cysteine may be essential for infants, the elderly, and individuals with certain metabolic diseases or who suffer from malabsorption syndromes (Lippard, *et al* 1994). Threonine is thus usually considered as glycine-like monoacid. However, the involvement of the oxygen atom of its hydroxy group in metal chelation can make it lose its proton at more accessible pH values (Freeman *et al.*, 1969, Grenouillet, *et al.*, 1973, Al Ani *et al.*, 1984)

Cetyltrimethylammonium bromide (CTAB) or cetrimonium bromide (or) hexadecyltrimethylammonium bromide is a cationic surfactant. It is one of the components of topical antiseptic cetrimide. Its uses include providing a buffer solution for the extraction of DNA. CTAB forms micelles in aqueous solution at 303K, with aggregation number 75-120 degree of ionization 0.2-0.1, the micelle size ( $r \approx 3$  nm). (The Merck Index, 2009)

## MATERIALS AND METHODS

### Experimental

#### Materials

0.05 mol dm<sup>-3</sup> aqueous solution of L-Cysteine and L-Threonine (GR grade, E-Merck, Germany) were prepared by dissolving samples in water. To increase the solubility of ligands, 0.05 mol dm<sup>-3</sup> hydrochloric acid concentration was

maintained in the solutions. The probable errors that may creep into the concentrations of the stock solutions of the ligands were determined by the computer program COSWT (Krishna Prasad, 1985). The pessimistic errors in the preparation of the ligand solutions by weight method did not exceed 0.1%. G R Sample of Cetyltrimethylammonium bromide (CTAB, Qualigens, India.) was used as such and its purity was checked by determining critical micellar concentration (CMC) conductometrically. CTAB, CMC value is 0.00093 M, at 303K. Sodium hydroxide of 0.4 mol L<sup>-1</sup> was prepared. The strengths of alkali and mineral acid were determined using the Gran plot method (Gran, 1952, Gran, 1988).

### Procedure

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 (Santhee Devi 2010). For the determination of protonation constants of Cys and Thr, 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<sup>-3</sup> with NaCl at 303K. In these titrations, the titrand consisted of

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mineral acid and ligand, in the presence and absence of metal ion, in a total volume of 50 cm<sup>3</sup>. Titrations were performed by adding each time 0.1cm<sup>3</sup> portions of sodium hydroxide (0.4 mol dm<sup>-3</sup>) 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 Titration Assembly

The glass electrode was equilibrated in well-stirred CTAB-water mixtures containing inert electrolyte for several days. At regular intervals titration of acid with alkali was carried out to check whether complete equilibration was achieved or not. Typical alkalimetric titrations are given in Fig. 1.

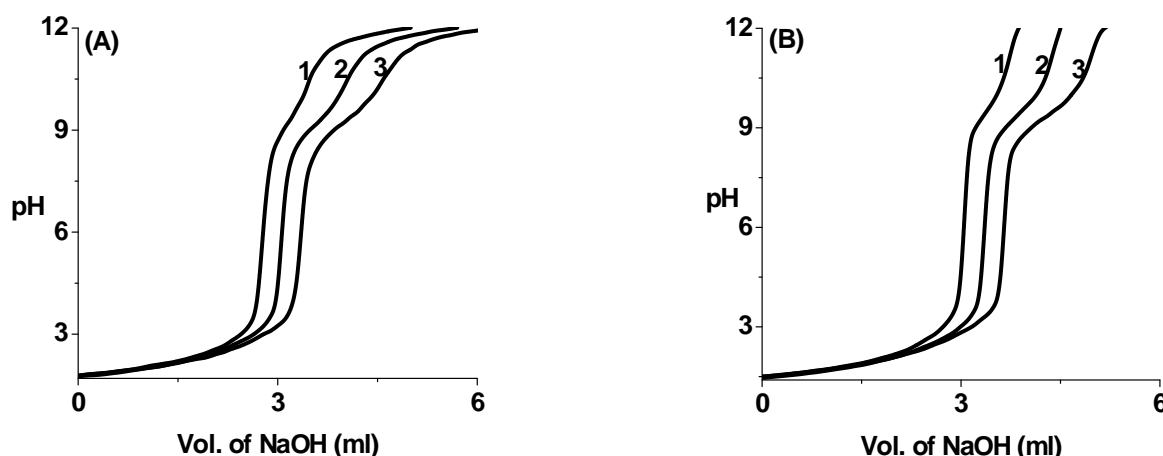


Fig 1 Alkalimetric titration curves of CTAB in 2.0% v/v (A) Cys and (B) Thr-water mixtures; 1, 2 and 3 indicate 0.25, 0.375 and 0.50mmol of ligand, respectively.

## RESULTS AND DISCUSSION

#### Modeling strategy

The approximate protonation constants of L-Cys and L-Thr were calculated with the computer program SCPHD (Rao, 1989) and they were refined using non-linear least-squares computer program MINQUAD75 (Gans, 1976). The variation of overall protonation constants were analyzed on electrostatic grounds on the basis of solute-solute and solute-solvent interactions. The best fit models that contain the type of species and overall protonation constants ( $\log \beta$ ) along with some of the important statistical parameters are given in Table 1.

A very low standard deviation in  $\log \beta$  values indicates the precision of these parameters. The small values of  $U_{\text{corr}}$  (sum of squares of deviations in the concentrations of ligand and hydrogen ion at all experimental points corrected for degree of freedom) indicate that the experimental data can be represented by the models. Small values of mean, standard deviation and mean deviation for the systems corroborate 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 as well as platykurtic patterns. The values of skewness are between -0.82 and 1.07.

These data evince that the residuals from a part of 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. The statistical parameters thus show that the best fit models portray the acido-basic equilibria of L-Cys and L-Thr in CTAB-water mixtures.

#### Effect of systematic errors

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

Table 1 Parameters of the best fit chemical models of protonation equilibria of L-Cys and L-Thr in CTAB-water mixtures at 303.0 K and ionic strength,  $\mu = 0.16 \text{ mol L}^{-1}$

% w/v	$\log \beta_{\text{mh}} \text{ (SD)}$			NP	$U_{\text{corr}} \times 10^8$	$\chi^2$	Skew-ness	Kurtosis	R-factor
	11	12	13						
Cys(pH range 2.0-10.0)									
0.0	6.38(11)	8.56(13)	10.67(11)	88	1.67	10.45	1.07	5.46	0.027553
0.5	6.83(4)	9.36(7)	11.28(5)	102	6.04	16.00	-0.82	8.22	0.028409
1.0	6.85(5)	9.45(6)	11.62(7)	114	4.47	6.53	-0.02	4.41	0.035294
1.5	6.46(5)	8.58(11)	10.86(6)	128	2.53	13.56	0.82	5.48	0.033943
2.0	6.38(11)	8.56(13)	10.67(11)	88	4.51	10.45	1.07	5.46	0.027559
2.5	6.85(6)	9.45(10)	11.62(7)	118	3.84	13.90	-0.58	5.06	0.038645
Thr(pH range 2.0-11.0)									
0.0	10.53(13)	18.48(28)	-----	33	1.39	2.00	-1.17	4.95	0.080021
0.5	11.49(8)	20.80(8)	-----	21	0.76	3.14	-1.63	6.05	0.005049
1.0	11.56(10)	20.65(10)	-----	46	1.08	10.29	0.16	3.21	0.040013
1.5	10.99(2)	20.28(1)	-----	39	1.68	25.38	-1.64	7.40	0.028288
2.0	15.54(7)	24.94(7)	-----	25	2.43	7.60	1.58	6.03	0.005682
2.5	11.56(10)	20.65(10)	-----	48	0.41	10.18	0.26	2.31	0.010004

$U_{\text{corr}} = U / (\text{NP} \cdot m)$ ; NP = Number of points; m = number of stability constants; SD = Standard deviation

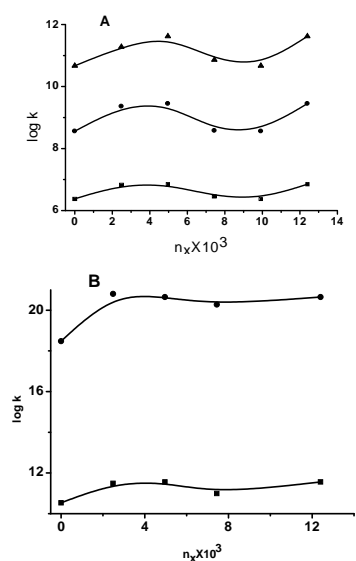
**Table 2** Effect of systematic errors in influential parameters on the protonation constants of L-Cysteine and L-Threonine in CTAB-water mixtures.in 1.5 w/v

Ingredient	% Error	log $\beta_{mn}$ (SD)				
		Cys			Thr	
		11	12	13	11	12
	0	6.38(11)	8.56(13)	10.67(11)	10.53(13)	18.48(28)
	-5	6.98(17)	8.45(29)	10.55(34)	10.76(31)	18.80(41)
	-2	6.74(10)	8.99(18)	10.02(22)	10.41(8)	18.17(12)
Alkali	+2	6.41(15)	8.40(28)	9.34(33)	10.02(11)	17.42(19)
	+5	6.16(25)	8.95(46)	10.83(55)	9.71(23)	17.86(39)
	-5	6.21(27)	8.94(50)	10.56(63)	9.86(20)	18.07(33)
	-2	6.44(16)	8.40(30)	10.24(36)	10.08(10)	18.51(17)
Acid	+2	6.72(9)	8.99(16)	11.11(19)	10.35(7)	18.08(12)
	+5	6.93(14)	9.43(25)	11.78(30)	10.57(23)	17.52(34)
	-5	6.52(11)	8.69(20)	10.82(24)	10.06(8)	18.57(14)
	-2	6.56(11)	8.69(20)	10.74(24)	10.15(6)	18.70(11)
Ligand	+2	6.60(11)	8.70(20)	10.63(24)	10.27(5)	18.87(9)
	+5	6.63(12)	8.90(21)	10.55(26)	10.35(6)	18.99(10)
	-5	6.17(25)	8.97(45)	10.89(54)	10.22(6)	18.80(11)
	-2	6.17(25)	8.96(46)	10.86(54)	10.22(6)	18.80(10)
log F	+2	6.16(25)	8.95(46)	10.81(55)	10.21(5)	18.78(9)
	+5	6.15(26)	8.94(46)	10.77(56)	10.20(5)	18.78(9)
	-5	6.16(25)	8.95(45)	10.92(53)	10.21(9)	18.78(15)
	-2	6.16(25)	8.95(45)	10.87(54)	10.21(6)	18.79(11)
Volume	+2	6.16(26)	8.95(46)	10.79(56)	10.21(5)	18.79(9)
	+5	6.16(26)	8.95(47)	10.73(58)	10.22(7)	18.80(12)

investigation was made by introducing pessimistic errors in the concentrations of alkali, mineral acid and the 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 more than those in the ligand and log F.

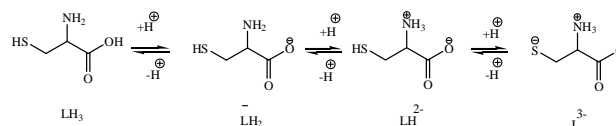
#### Effect of solvent

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 (Born, 1920) 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 logK values in present study are linearly increasing (Fig. 2) with decreasing dielectric constant of the medium in both the Ligands (Cys and Thr).

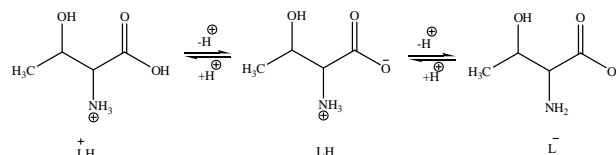


**Fig 2** Variation of stepwise protonation constant (log K) with mole fraction of solvent.(A)Cys and (B) Thr in ctab-water mixtures ; (■) log  $K_1$ , (●) log  $K_2$ .

L-Cysteine exists as anion (L<sup>-</sup>), zwitter ion (LH) and cation (LH<sub>2</sub><sup>+</sup>) and (LH<sub>3</sub><sup>2+</sup>) (Fig. 3) at different pH values. L-Threonine exists as anion (L<sup>-</sup>), zwitter ion (LH) and cation (LH<sub>2</sub><sup>+</sup>) (Fig. 4) at different pH values. The cation stabilizing nature of co-solvent, specific solvent-water interactions, charge dispersion and specific interactions of co-solvent with solute account for the linear relationship of log K with mole fraction of solvent.



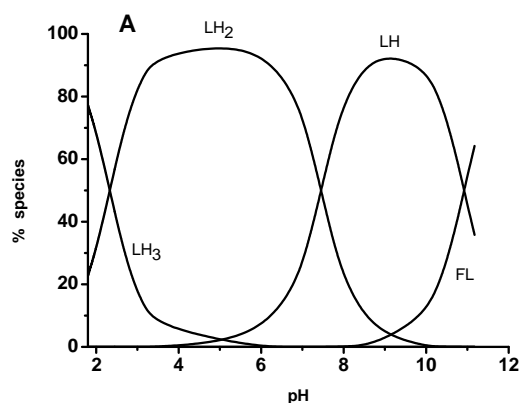
**Fig 3** Protonation-deprotonation equilibria of L-Cysteine



**Fig 4** Protonation-deprotonation equilibria of L-Threonine

#### Distribution Diagrams

L-Cysteine exists as anion (L<sup>-</sup>), zwitter ion (LH) cation and (LH<sub>3</sub><sup>2+</sup>) (Fig. 3 A) at different values.



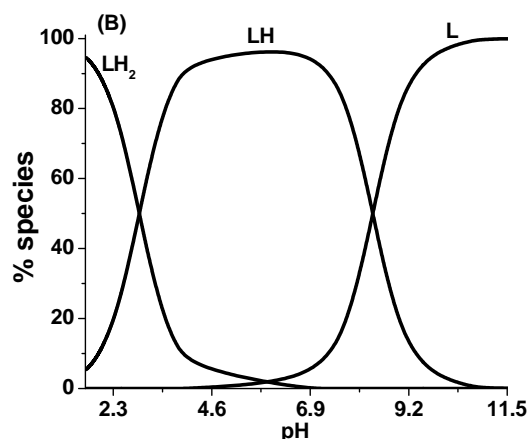


Fig 4 distribution diagrams of L-Cys in 0.5% w/v (A), L-Thr in 0.5% w/v(B) in CTAB- water mixture.

(LH<sub>3</sub><sup>2+</sup>) exists at pH 2.0-5.0, (LH<sub>2</sub><sup>+</sup>) exists at pH 2.0-9.0, (LH) exists at pH 6.0-11.0 and (L<sup>-</sup>) exists pH 9.0-11.6. L-Threonine exists as anion (L<sup>-</sup>), zwitter ion (LH) and cation (LH<sub>2</sub><sup>+</sup>) (Fig. 4) at different pH values. (LH<sub>2</sub><sup>+</sup>) exists at pH 2.0-6.0, (LH) exists at pH 2.0-11.0 and (L<sup>-</sup>) exists at pH 6.0-11.5.

## CONCLUSIONS

1. L-Cys exists as LH<sub>3</sub><sup>2+</sup> at low pH and gets deprotonated with the formation of LH<sub>2</sub><sup>+</sup> and LH successively with increase in pH. L-Thr exists as LH<sub>2</sub><sup>+</sup> at low pH and gets deprotonated with the formation and LH successively
2. The log K values of protonation constants increase linearly with decreasing dielectric constant of Cys and Thr in CTAB-water mixtures. This trend indicates the dominance of electrostatic forces over the non-electrostatic forces in the protonation-deprotonation equilibria
3. The effect of systematic errors in the influential parameters shows that the errors in the concentrations of alkali and mineral acid affect the protonation constants more than that of the ligand and log F.

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