

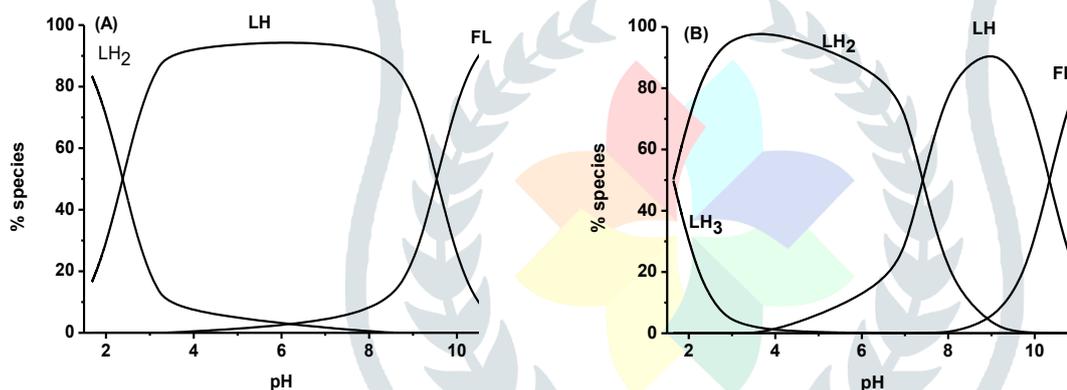
# SOLVENT EFFECT ON PROTONATION EQUILIBRIA OF L-METHIONINE AND L-CYSTEINE IN NEUTRAL SURFACTANT MEDIUM

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**Abstract:** The impact of Triton X-100 on the protonation equilibria of L-methionine and L-cysteine has been studied in various concentrations (0.5-2.5% v/v) of TX-100 solution maintaining an ionic strength of 0.16 mol dm<sup>-3</sup> at 303K using a pH-metric method. The protonation constants have been calculated using the computer program MINIQUAD75 and the best fit models are arrived based on statistical grounds employing crystallographic R factor,  $\chi^2$ , skewness 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. 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. Distribution of species, protonation equilibria and effect of influential parameters on the protonation constants has also been presented.



Species distribution diagrams of (A) Met and (B) Cys in 1.0% v/v Triton X-100 water mixture

**Indexterms-** Protonation equilibria, Triton X-100, L-methionine, L-cysteine, MINIQUAD75.

## I. INTRODUCTION

**L-Methionine:** (Met) Methionine and cysteine may be considered to be the principal sulfur-containing amino acids because they are 2 of the canonical 20 amino acids that are incorporated into proteins. Both methionine and cysteine play critical roles in cell metabolism. Met is among the most hydrophobic of the amino acids. Most of the methionine residues in globular proteins are found in the interior hydrophobic core; in membrane-spanning protein domains. Met is often found to interact with the lipid bilayer. Met also belongs to a group of compounds called lipotropics, or chemicals that help the liver process fats (lipids). Levine et al. [1] regard these methionine residues as endogenous antioxidants in proteins.

**L-Cysteine:** (Cys) Cysteine is a naturally occurring, sulfur-containing amino acid that is found in most proteins in small quantities. The sulfur atom of cysteine is involved in formation of the sulfhydryl group which is very reactive. Cys is an important amino acid which, when protonated at low pH, has three acidic hydrogens: carboxyl ( $-\text{COOH}$ ), amino ( $-\text{NH}_3^+$ ), and thiol ( $-\text{SH}$ ): Cys is 'biogenic' amino acid which is linked by peptide bonds form polypeptides and proteins. Cys is abundant as L-form. Cys can be easily oxidized to form a dimer containing disulfide bridge between two cysteines. Such dimer is known as cystine. Cys is a precursor to glutathione, which is instrumental in protecting against exposure to environmental toxins, especially heavy metals. Cys play a role in the treatment of cobalt toxicity, diabetes, psychosis, cancer and seizures.

**Triton X-100 :** It is a non-ionic surfactant that has a hydrophilic polyethylene oxide chain (on average it has 9.5 ethylene oxide units) and an aromatic hydrocarbon lipophilic or hydrophobic group. The hydrocarbon group is a 4-(1,1,3,3-tetramethylbutyl)-phenyl group. Triton X-100 is a commonly used detergent in laboratories [2]. Triton X-100 (TX100) is one of the most widely used non-ionic surfactants for lysing cells to extract protein and other cellular organelles or to permeabilize the living cell membrane for transfection (3–5). However, if large amounts are added or the cells are subject to prolonged exposure to TX100, the cells die (6–9). This toxicity of TX100 molecules arises because of the disrupting action of its polar head group on the hydrogen bonding present within the cell's lipid bilayer, leading to the destruction of the compactness and integrity of the lipid membrane. The insertion of detergent monomer into the lipid membrane begins at low concentrations. This leads to a disruption of cellular structure

and eventual over permeabilization of the cell membrane at concentrations above the critical micelle concentration (CMC) from the bilayer–micelle transition. All members of this family (Triton X100, Triton X114, Nonidet P40 [NP-40], Igepal® CA-630) are quite similar and differ only in their average number ( $n$ ) of monomers per micelle (9.6, 8.0, 9.0, and 9.5, respectively) and in the size distribution of the PEG-based head group [10].

Solvent effect on protonation equilibria of L- Methionine and L-Cysteine in Triton X-100-water mixtures has been investigated. An insight into the protonation equilibria is also helpful in understanding the metal-ligand equilibria associated with these ligands.

## II. EXPERIMENTAL

### 2.1 Materials

Solutions of 0.05M L- Met (Hi-media), 0.05M L-Cys (Hi-media), 0.2M Hydrochloric acid (Merck, India), 0.4M of sodium hydroxide (Merck, India) and Triton X-100 (Hi-media) were prepared in triple-distilled deionised water. Sodium chloride (Merck, India) of 2.0 M was prepared to maintain the ionic strength in the titrand. The acid and base solutions were standardised 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 was determined using the standardised 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 COST (Rao *et al.*, 2005). The strength of the prepared carbonate-free sodium hydroxide solution was determined by titrating it against hydrochloric acid solution using the Gran plot method (Gran, 1988)

### 2.2. Alkalimetric titrations

The pH measurements of the proton–ligand system were carried out in aqueous media containing varying compositions of surfactant (TX-100) in the range of 0.5–2.5% v/v maintaining an ionic strength of 0.16 M with sodium chloride at  $303.0 \pm 0.1$  K using a Digital pH meter ELICO-LI120 type (readability 0.01). Potassium hydrogen phthalate (0.05 M) and borax (0.01 M) solutions were used to calibrate the pH meter. In each titration, the titrand consisted of approximately 1 mmol of hydrochloric acid. The amounts of the Met, Cys (ligands) in the titrand are in the range of 0.25–0.50 mmol. The glass electrode was equilibrated in a well stirred surfactant–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 experimental procedure and titration assembly used in our laboratory have been given elsewhere (Latha *et al.*, 2007).

### 2.3 Modeling Strategy

The approximate protonation constants of Met and Cys were calculated with the computer program SCPHD [11] and from the secondary formation functions. The data were pruned using a non-linear least-squares computer program MINQUAD75 [12]. The reliability of the protonation constants were verified from the statistical parameters and by introducing errors in the concentrations of the ingredients. The best set of protonation constants were selected based on chemical validation.

## III. Results and Discussion

The best fit model having the type of species and protonation constants together with certain statistical parameters are given in table I. The values of low standard deviation (SD) in  $\log \beta$  and  $U_{\text{corr}}$  (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. For an ideal normal distribution, the values of kurtosis and skewness should be three and zero, respectively. The values of kurtosis in table 1 signify that the residuals form leptokurtic patterns. The skewness values (-0.05 and 0.42) 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 Met and Cys in neutral surfactant medium.

Table I: BEST FIT CHEMICAL MODELS OF PROTONATION EQUILIBRIA OF L-METHIONINE AND L-CYSTEINE IN TRITON X-100-WATER MIXTURES Temp= 303 K, Ionic strength=0.16 mol dm<sup>-3</sup>.

% v/v TX-100	logβ <sub>1</sub> (SD)	log β <sub>2</sub> (SD)	log β <sub>3</sub> (SD)	NP	U <sub>corr</sub> ×10 <sup>8</sup>	Skewness	Kurtosis	χ <sup>2</sup>	R-factor
<b>METHIONINE (pH range 1.60-10.50)</b>									
0	8.93(07)	11.17(09)	---	54	26.23	0.42	5.45	5.93	0.0410
0.5	9.51(05)	11.47(06)	---	76	21.25	-0.35	2.95	27.16	0.0222
1.0	9.54(05)	11.92(06)	---	76	22.10	-0.46	2.48	7.37	0.0229
1.5	9.53(07)	11.82(11)	---	33	38.30	-0.10	2.50	2.61	0.0555
2.0	9.60(05)	12.42(07)	---	53	18.55	-3.23	20.24	20.49	0.0298
2.5	9.77(05)	12.53(07)	---	48	18.56	-0.05	3.25	9.33	0.0299
<b>CYSTEINE (pH range 2.0-10.80)</b>									
0	10.53(02)	18.67(01)	20.53(09)	60	14.13	-0.83	6.41	22.67	0.0241
0.5	10.25(05)	17.74(04)	19.56(16)	70	19.71	-0.91	2.48	38.51	0.0586
1.0	10.34(04)	17.85(03)	19.39(11)	91	73.54	-0.82	2.70	51.49	0.0477
1.5	11.49(03)	17.97(02)	20.67(17)	69	55.98	-0.73	4.20	35.91	0.0525
2.0	10.27(03)	17.90(01)	19.85(02)	103	76.38	0.39	3.38	27.01	0.0372
2.5	11.07(03)	18.32(02)	20.88(11)	63	46.35	-0.61	2.16	31.59	0.0439

U<sub>corr</sub>=U/(NP-m), where m=number of species; NP=Number of experimental points; SD=standard deviation

### 3.1 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 of the study corresponds to the number of equilibria. Two half integrals (0.5 and 1.5) in the case of Met (Figure 1A) emphasise the presence of two protonation deprotonation equilibria in the pH range of present study. Three half integrals (0.5, 1.5, and 2.5) versus pH in the case of Cys (Figure 1B) confirm the presence of three protonation–deprotonation equilibria. The maximum value of Met and Cys in the formation curve of (Figure-1A and 1B) is two and three respectively which clearly shows that has Met and Cys two, three bound protons per molecule in the pH range of present study. The plots of  $\bar{n}_H$  versus pH are given in Figure 2. The negative values of  $\bar{n}_H$  correspond to the number of moles of free acid present in the titrand and the number of associable protons. The positive values of  $\bar{n}_H$  indicate the number of dissociable protons in the ligand molecules. The maximum value of  $\bar{n}_H$  in Figure 2(A) is +1, which indicates that L- Met has one dissociable carboxyl proton. The corresponding value for Cys in Figure 2(B) is 2, which clearly infers that Cys has two dissociable protons one carboxyl proton and one from HS proton.

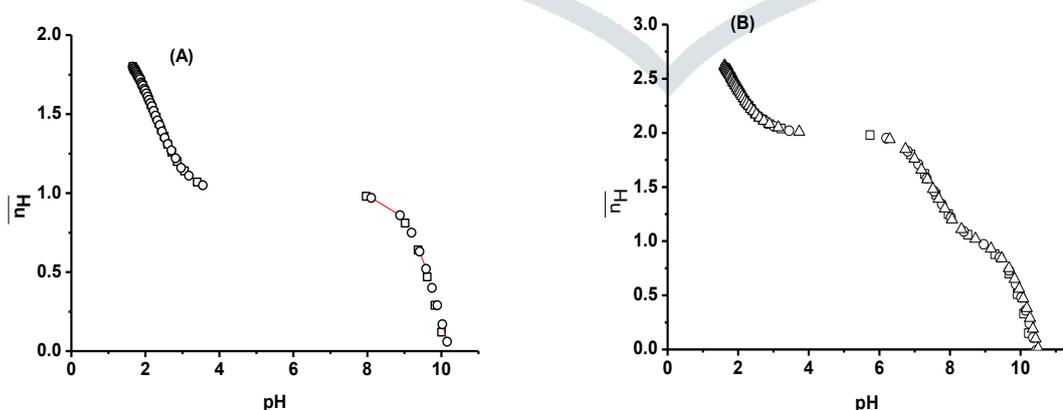


Figure 1: Plots of  $\bar{n}_H$  versus pH of (A) Met and (B) Cys: (□) 0.25, (○) 0.375, and (Δ) 0.50 mmol, respectively. in 0.5% v/v Triton X-100 water mixture respectively

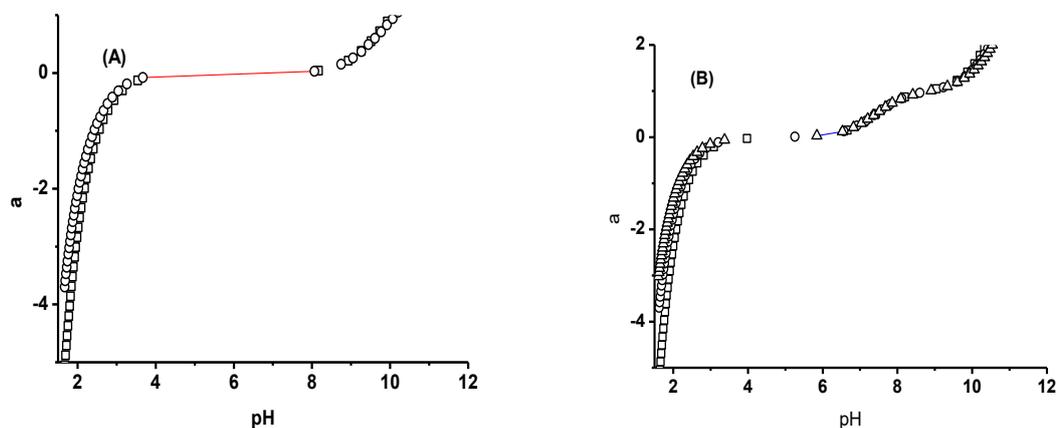


Figure 2: Variation of  $a$  versus pH in 1.0 % v/v TritonX-100 water mixture; of (A) Met and (B) Cys(  $\square$ ) 0.25, ( $\circ$ ) 0.375, and ( $\Delta$ ) 0.50 mmol, respectively. In 1.0% v/v Triton X-100 water mixture respectively.

### 3.2 Distribution Diagrams

Typical distribution plots produced by DISPLOTT [13] using protonation constants from the best fit models are shown in Figure 3. Representative plots show the existence of LH<sub>2</sub>, LH, FL in the case of Met and LH<sub>3</sub>, LH<sub>2</sub>, LH in the case of Cys in 2.0-10.0 pH ranges. LH form of Met is present to an extent of 90% in the pH range 3.0-9.0 and LH<sub>2</sub> form of Cys is present to an extent of 90% in the range 3.0-6.0. The present study is useful to understand (i) the role played by the active site cavities in biological molecules, (ii) the type of complex formed by the metal ion and (iii) the bonding behaviour of the protein residue with the metal ion. The species refined and the relative concentrations under the present experimental conditions represent the possible forms of these amino acids in the biological fluids.

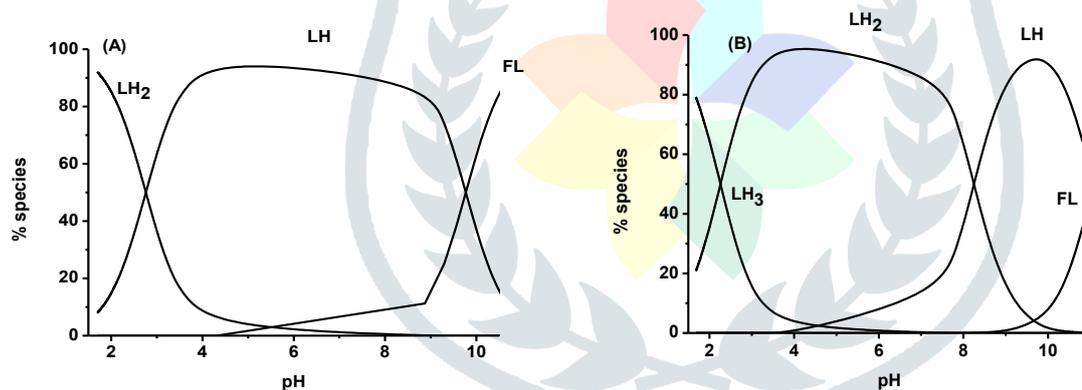


Figure3: Species distribution diagrams of (A) Met and (B) Cys in 2.5% v/v TritonX-100-water mixture

### 3.3 Effect of systematic errors on best fit model:

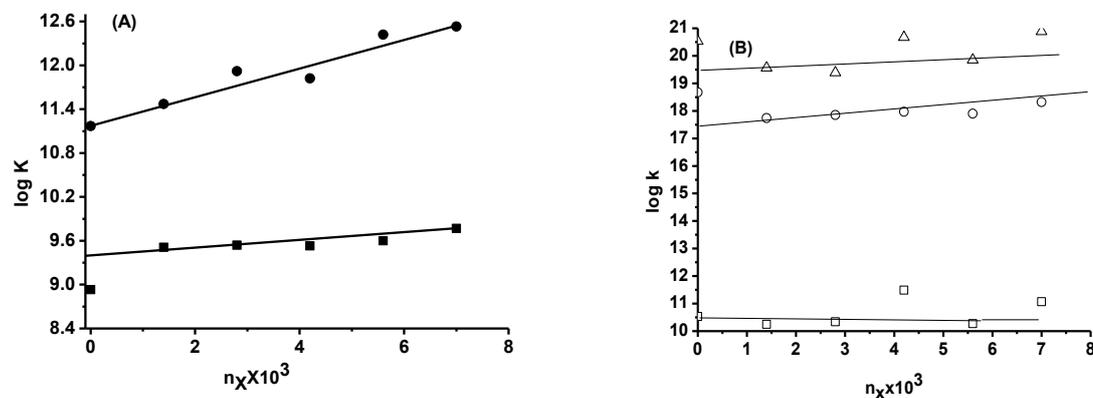
Any variation in the concentrations of ingredients like alkali, mineral acid and ligand affects the magnitudes of protonation constants. Such parameters are called influential or dangerous parameters. The results of a typical system given in Table II emphasize that the errors in the concentrations of alkali and mineral acid affect the protonation constants more than that of the ligand.

Table II Effect of systematic errors in influential parameters on the protonation constants of met and cys in 1.0% v/v triton x-100-water mixture

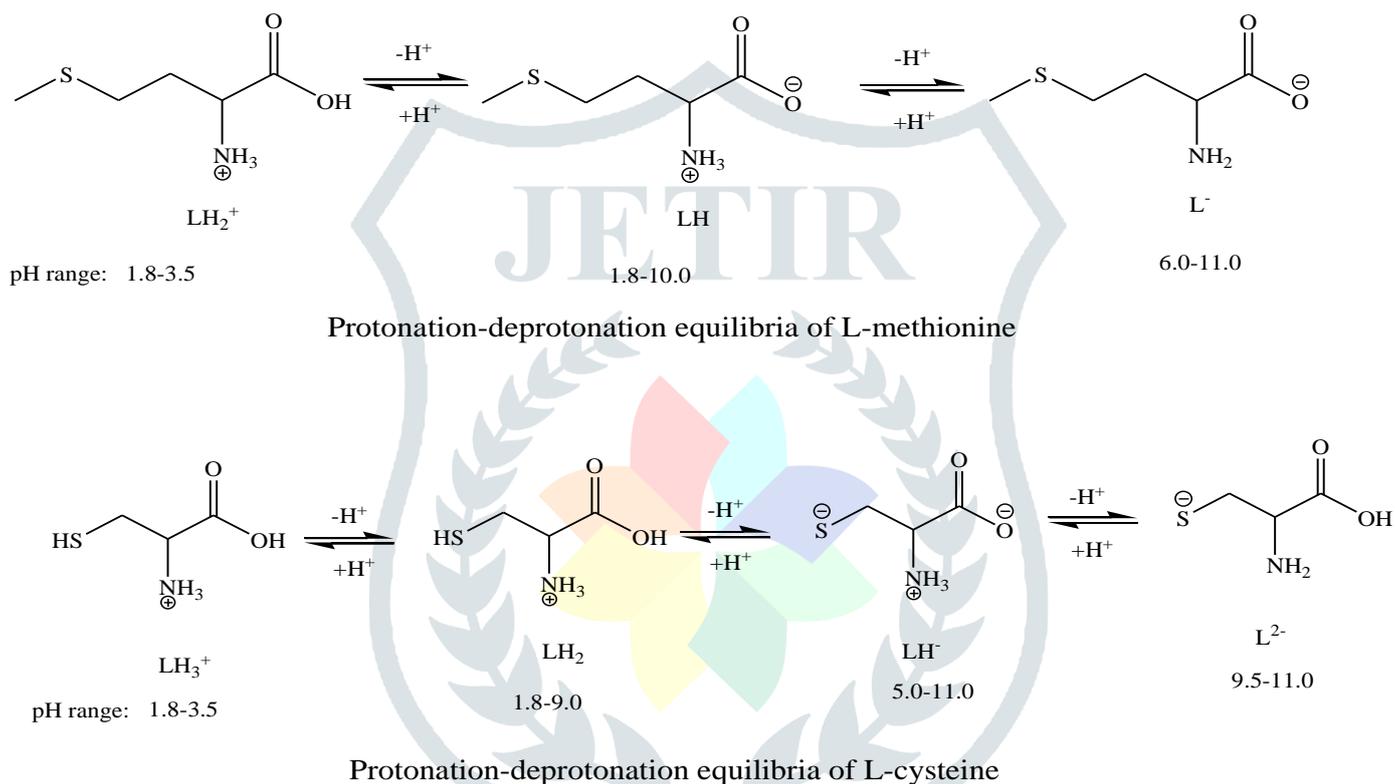
Ingredient	% Error	log $\beta_{mlh}$ (SD)				
		Met		Cys		
		11	12	11	12	13
	0	9.54(05)	11.92(06)	10.34(04)	17.85(03)	19.39(11)
Alkali	-5	9.87(13)	11.34(05)	Rejected	17.98(18)	19.45(12)
	-2	Rejected	11.56(04)	10.56(05)	17.45(02)	19.10(14)
	+2	9.34(15)	11.77(16)	10.87(15)	Rejected	19.87(10)
	+5	9.95(32)	Rejected	11.05(18)	18.06(21)	19.67(08)
Acid	-5	9.66(32)	12.10(14)	10.45(23)	17.87(43)	Rejected
	-2	9.45(34)	11.86(22)	Rejected	17.56(34)	19.55(33)
	+2	Rejected	12.23(32)	10.66(18)	17.98(28)	19.86(24)
	+5	10.15(24)	Rejected	10.98(19)	18.04(06)	20.16(22)
Ligand	-5	9.66(16)	12.05(32)	10.56(25)	17.98(32)	19.78(19)
	-2	9.34(25)	11.78(35)	10.76(08)	18.02(15)	19.34(18)
	+2	9.45(32)	11.54(17)	10.88(17)	17.93(15)	19.98(35)
	+5	9.88(16)	11.78(19)	10.75(43)	18.05(26)	20.17(16)

### 3.4 Effect of Micelles :

TritonX-100 is a non-ionic surfactant. In this medium the charged species are expected to be destabilised. The protonation of neutral ligands like phenols[14] and carboxylic acids has increased due to the destabilisation of the charged phenolate and carboxylate ions. Aniline has decreased protonation constants because of the destabilisation of the charged anilinium cation in the low-polarity medium of the micelle. Always such a simple trend need not be observed in non-ionic micellar media as electrostatic factors are relatively less important compared to the non-electrostatic forces. The effect of surfactant on protonation equilibria was recognised long back [15]. The apparent shift in the magnitude of protonation constants in micellar media compared to aqueous solutions was attributed to the creation of a concentration gradient of protons between the interface and the bulk solutions [16]. The presence of micelles is known to alter the dielectric constant of the medium, which has a direct influence on the protonation-deprotonation equilibria [17-19]. The variation of protonation constants with solvent depends upon two factors, viz., electrostatic and non-electrostatic interactions. Born's classical treatment holds good in accounting for the electrostatic contribution to the free energy change [20]. According to this treatment, the energy of electrostatic interaction or the logarithm of step-wise protonation constant (log K) should vary linearly as a function of the mole fraction of the medium. The log K values in the present study are linearly increasing (Figure 4) with increasing mole fraction of the medium in both the amino acids.



**Figure 4:** Variation of stepwise protonation constant (log K) with mole fraction of TritonX-100 in TritonX-100 –water mixtures. (A) L- Met (□) logK<sub>1</sub>, (○) logK<sub>2</sub>, (B) L-Cys (□) logK<sub>1</sub>, (○) logK<sub>2</sub>, (Δ) logK<sub>3</sub>.



#### IV. CONCLUSIONS

1. L-Methionine forms  $LH_2^+$  at low pH and gets deprotonated with the formation of  $LH$  and  $L^-$  respectively with increase in pH.
2. Cysteine forms  $LH_3^+$  at low pH and gets deprotonated with the formation of  $LH_2^+$ ,  $LH$  and  $L^{2-}$  respectively with increase in pH.
3. The change of log values of protonation constants of L- Methionine and L-Cysteine linearly increases with increasing mole fraction of CTAB-water mixtures which indicate the dominance of electrostatic forces in the protonation-deprotonation equilibria and hydrogen bonding.
4. Alteration in the concentrations of components like acid and ligand affects the protonation constants more than that of the change in alkali.

#### REFERENCES

- [1] Levine R.L, Mosoni L, Berlett B.S, Stadtman E.R. 1996. Methionine residues as endogenous antioxidants in proteins. Proc Natl Acad Sci USA. 93,15036–40
- [2] Johnson M. 2018. Detergents: Triton X-100, Tween-20, and More. Materials and Methods. 3,163-72.
- [3] Gennuso F, et al. 2004 Bilirubin protects astrocytes from its own toxicity by inducing up-regulation and translocation of multidrug resistance-associated protein 1 (mrp1). Proc Natl Acad Sci USA. 101,2470–2475.
- [4] Rajagopal A, Pant AC, Simon SM, Chen Y. 2002 In Vivo analysis of human multidrug resistance protein (MRP1) activity using transient expression of fluorescently Tagged MRP1. Cancer Res. 62,391–396
- [5] Hipfner DR, Gaudie SD, Deeley RG, Cole SPC. 1994. Detection of the *M<sub>r</sub>* 190,000 multidrug resistance protein, MRP, with monoclonal antibodies. Cancer Res. 54, 5788–5792.
- [6] Borner MM, et al. 1994. The detergent TX100 induces a death pattern in human carcinoma cell lines that resembles cytotoxic lymphocyte-induced apoptosis. FEBS Lett. 353,129–132.

- [7] Benoit J, Cormier M, Wepierre J. 1988. Comparative effects of four surfactants on growth, contraction and adhesion of cultured human fibroblasts. *Cell Biol Toxicol.* 4(1),111–122.
- [8] Dayeh VR, Chow SL, Schirmer K, Lynn DH, Bols NC. 2004. Evaluating the toxicity of TX100 to protozoan, fish, and mammalian cells using fluorescent dyes as indicators of cell viability. *Ecotox Environ Safe.* 57,375–382.
- [9] Laouar L, Lowe KC, Mulligan BJ. 1996 Yeast response to nonionic surfactants. *Enzyme Microb Tech.* 18,433–438
- [10] Arnold T, Linke D. 2007. Phase separation in the isolation and purification of membrane proteins. *Biotechniques.* 43,427-30, 432, 434 p
- [11] Rao G.N. 1989. Complex equilibria of some biologically important metal ions in aquo-organic media. Ph. D. Thesis. Andhra University, Visakhapatnam, India..
- [12] Gans P, Sabatini A, Vacca A. 1976. An improved computer program for the computation of formation constants from potentiometric data. *Inorg. Chim. Acta.* 18, p237-239,
- [13] Rao G.N, Babu A.R, Satyanarayana S.V.V, Satyanarayana A, Rao RS and Ramana KV. DISPLOT-A computer program for distribution plots of proton-ligand and unprotonated metal ligand complexes in solution phase. *Acta Cienc Indica.* 15, 321-338
- [14] TONG L.K.J, GLESSMANN M.C, 1957 . The Mechanism of Dye Formation in Color Photography. The Effect of a Non-ionic Surfactant on the Ionization of Couplers. *J. Am. Chem. Soc.,* 79, 4305 (1957).
- [15] Hartly G S, *Trans Faraday Soc,* 30(1934) 444.
- [16] Hartly G.S, Roe J.W.1940. *Trans Faraday Soc,* 36 ,101.
- [17] Bunton C.A, *Catal. Rev. Sciencz.,* 1979. 20.
- [18] Bunton C.A, Romsted L.S, Supulveda L, 1980. *J. Phys. Chem.,* 84 ,2611.
- [19] Chaimovich H, Politi M.J, Bonilha J.B.S, Quina F.S, 1979. *J. Phys. Chem.,* 83 ,1857.
- [20] Born M, et,al. 1920. *Phys.,* 1,45.

