# Influence of Dielectric constant on Protonation Equilibria of L-Cysteine and L-Methionine in 1, 4-Dioxane-Water Mixture

**K. Bharath Kumar Naik**
*Department of Chemistry*
*Dr. V. S. Krishna Govt. Degree & PG College(A), Maddilapalem Visakhapatnam-531013, Andhra Pradesh-India*

## Abstract

The solute-solvent interactions of L-Cysteine and L-Methionine have been studied pH metrically in various concentrations (0.0-60.0% v/v) 1, 4-Dioxane-water mixture maintaining an ionic strength of 0.16 mol L-1 at ambient conditions. The protonation constants have been calculated with the computer program MINIQUAD75 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. Protonation equilibria and effect of influential parameters on the protonation constants have also been presented.

**Keywords- Protonation equilibria, L-Cysteine, L-Methionine, 1, 4-Dioxane**

## I. INTRODUCTION

Cysteine(Cyst) is the one of the sulfur containing non-essential amino acids along with methionine, which are found naturally in many proteins such as α and β-ceratins, and in free state. The ability of Cyst residues to form disulfide bridges plays an important role on secondary structure and folding of proteins. Another prominent quality is that sulfur can be found in different oxidation states in living systems, which is a key for various biological functions. More about the role and importance of sulfur containing amino acids as well as redox chemistry of Cyst residues in enzymes is summarized in [1, 2].

All plants and some forms of bacteria can synthesize Methionine (Met) by two different sequences [3]. Mammalian tissues and many bacteria synthesize it from cobalamine enzyme whereas all the plants and some bacteria synthesize it from homocysteine. Cerebrospinal fluid levels of Met, homocysteine and cystathionine were studied in patients with psychotic disorders [4]. Met is synthesized from cysteine and o-phosphohomoserine involving three enzymes, cystathionine γ -synthase, cystathionine β-lyase and methionine synthase [5].

Dioxane (DOX) is an aprotic solvent capable of acting as hydrogen bond acceptor with random structure [6]. The DOX-water mixtures are the combination of aprotic and protic solvents with wide range of dielectric constants and with good solubility for polar as well as non-polar solutes. The co-solvent-induced increased basicity of DOX-water mixtures increases the stabilization of protons. The dielectric constant of the medium decreases with increase in the mole fraction of the DOX. Hence, this medium is chosen to study the acido-basic equilibria to mimic the physiological conditions where the concept of equivalent solution dielectric constant for protein cavities is applicable [7]. A number of studies have been reported on protonation constants of α-amino acids in different media [8-10]. Acidity and basicity of a molecule is governed by its structure and solvent effects [11, 12]. A review of literature has revealed that a little is reported for protonation constants of Cysteine and Methionine in low dielectric media. The present study reveals the determination of protonation constants of Cysteine and Methionine in 1, 4-Dioxane-water mixture.

## II. EXPERIMENTAL

### *A. Materials*

0.05 mol $L^{-1}$ solutions of L-Cysteine (Cyst) and L-Methionine (Met) (Sigma-Aldrich, Germany) was prepared in triple distilled water by maintaining 0.05 mol $L^{-1}$ acid (HNO3) concentration to increase the solubility. 1, 4-Dioxane (AR, E-Merck) was used as received. Sodium nitrate was prepared to maintain the ionic strength in the titrand. Sodium hydroxide of 0.4 mol $L^{-1}$ was prepared. The strengths of alkali and mineral acid were determined using the Gran plot method [13, 14].

### *B. Procedure*

The titrimetric data were obtained by using calibrated SYSTRONIC (Microprocessor Model 361) 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 [15]. For the determination of protonation constants of Cyst and Met, 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. All the titrations have been carried out in a medium containing varying concentrations of solvent (0.0-60.0% v/v) and maintaining an ionic strength of 0.16 mol $L^{-1}$ with sodium nitrate at ambient conditions. In each of the titrations, the titrand consisted of approximately 1 mmol mineral acid in a total volume of 50 ml. Titrations with different volumes (2.50, 3.75 and 5.00 ml) of ligand were carried out with 0.4 mol $L^{-1}$ sodium hydroxide. Other experimental details are given elsewhere [16].

### *C. Alkalimetric Titration Assembly*

The glass electrode was equilibrated in a well stirred DOX - 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 Figure 1.

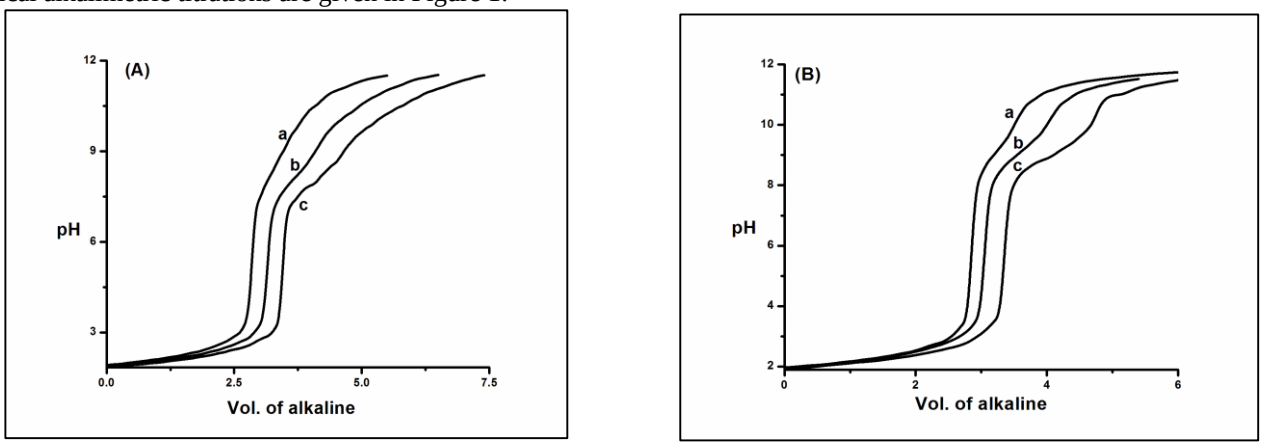


Fig. 1: Alkalimetric titration curves of (A) Cysteine and (B) Methionine in 50% v/v Dioxan- water mixtures; a, b, and c indicate 0.250, 0.375 and 0.500 mmol of ligand respectively

## III. RESULTS AND DISCUSSION

### *A. Modeling strategy*

The approximate protonation constants of Cyst and Met were calculated with the computer program SCPHD [17] and they were refined using non-linear least-square computer program MINIQUAD75 [18]. 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 β) along with some of the important statistical parameters are given in Table 1.

*Table 1: Parameters of best-fit chemical models of protonation equilibria of Cysteine and Methionine in DOX-water mixtures at ambient conditions and ionic strength, $\mu = 0.16$ mol $L^{-1}$*

| % v/v DOX | $Log\beta_{mlxh}(SD)$ | | | NP | $U_{corr}$ X $10^8$ | Skew-ness | Kurto-sis | $\chi^2$ | R-Factor |
|---|---|---|---|---|---|---|---|---|---|
| | $LH_3$ | $LH_2$ | LH | | | | | | |
| L – Cysteine | | | | | | | (pH range: 1.75 - 10.60) | | |
| 0 | 10.47(2) | 18.90(2) | 21.21(3) | 125 | 05.2623 | -.45 | 4.10 | 11.95 | .0096 |
| 10 | 10.49(3) | 18.90(5) | 21.27(6) | 131 | 21.3178 | .38 | 4.13 | 15.68 | .0199 |
| 20 | 10.52(5) | 18.92(7) | 21.35(9) | 118 | 31.6522 | .79 | 3.94 | 9.90 | .0237 |
| 30 | 10.57(3) | 18.93(6) | 21.46(7) | 104 | 22.1782 | .10 | 2.35 | 18.77 | .0220 |
| 40 | 10.63(3) | 18.95(4) | 21.62(6) | 92 | 13.4831 | .07 | 3.46 | 10.26 | .0169 |
| 50 | 10.72(2) | 18.98(2) | 21.84(3) | 89 | 03.8837 | -.32 | 7.94 | 50.94 | .0099 |
| 60 | 10.84(3) | 19.02(4) | 22.22(5) | 102 | 06.5050 | -.18 | 4.61 | 8.55 | .0119 |
| L – Methionine | | | | | | | (pH range: 1.75 - 9.20) | | |
| 0 | 9.25(1) | 11.57(2) | --- | 119 | 07.0941 | .80 | 20.18 | 25.26 | .0122 |
| 10 | 9.18(5) | 11.63(6) | --- | 79 | 23.3766 | .01 | 5.63 | 39.39 | .0275 |
| 20 | 9.10(3) | 11.70(4) | --- | 78 | 22.6315 | .08 | 2.66 | 10.87 | .0283 |
| 30 | 9.02(3) | 11.78(4) | --- | 85 | 21.6867 | .24 | 2.71 | 6.28 | .0265 |
| 40 | 8.95(2) | 11.86(3) | --- | 70 | 10.4117 | .44 | 4.16 | 25.37 | .0206 |
| 50 | 8.87(1) | 11.95(2) | --- | 69 | 04.5821 | -.11 | 4.31 | 4.61 | .0137 |
| 60 | 8.68(2) | 12.16(3) | --- | 74 | 49.6389 | .49 | 3.29 | 11.68 | .0177 |

A very low standard deviation in log β values indicates the precision of these parameters. The small values of $U_{corr}$ (sum of the squares of deviations in 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 -0.45 and 0.80. 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 Cyst and Met in DOX- water mixtures.

### *B. 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 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.

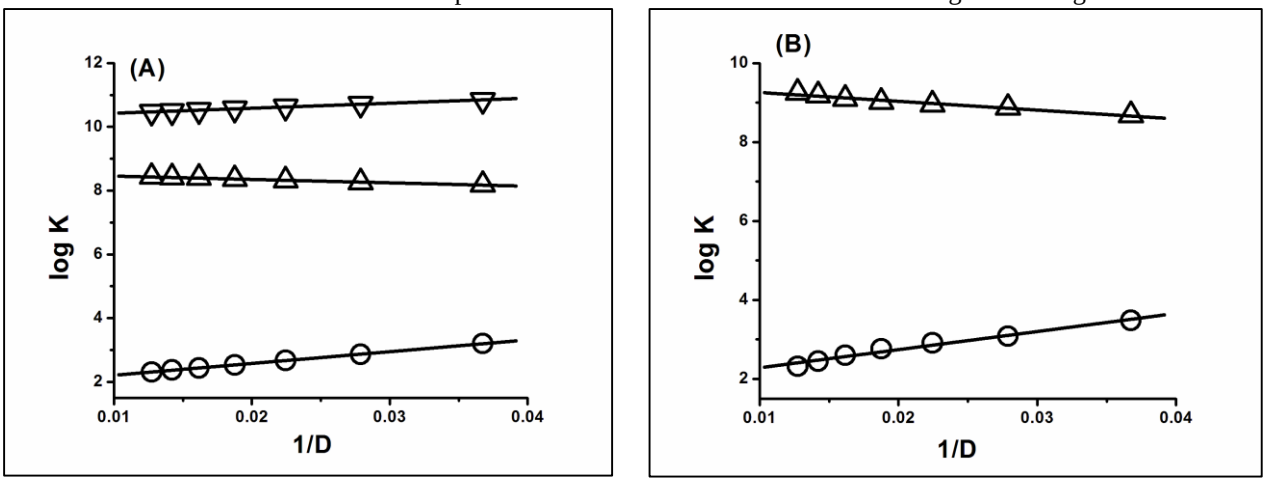


Fig. 2: Variation of stepwise protonation constant of (logK) with reciprocal of dielectric constant (1/D) of solvent. (A) Cyst and (B) Met in DOX- water mixtures; (O) log $K_1$, (Δ) log $K_2$ and (∇) log $K_3$

### *C. 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 [19] 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. In present study the log K values are linearly increasing (Figure 3) with decreasing dielectric constant of the medium. In figure 3B the log K values are linearly decreasing gap of between the two stability constants are decreasing with DOX concentration. This linearly decreases the dominance of the structure-forming nature of water and co-solvent and solvent-solvent interactions with the progressive increase of co-solvent content.

### *D. Cysteine and Methionine*

Cyst exists as cation ($LH_3^+$), zwitterions ($LH_2$) and anions ($LH^-$ and $L^{2-}$) and Met exists as cation ($XH_2^+$), zwitterions (XH) and anion ($X^-$) (Figure 5) 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 1/D.

### *E. Distribution Diagrams*

Secondary formation function ($\bar{n}_H$) average number of moles of protons bound per mole of ligand is useful to detect the number of protonation equilibria. The formation functions (Figure 4) indicate that Cysteine has three and Methionine has two equilibria. The pH values at half integrals of $\bar{n}_H$ correspond to the log K values of the ligands

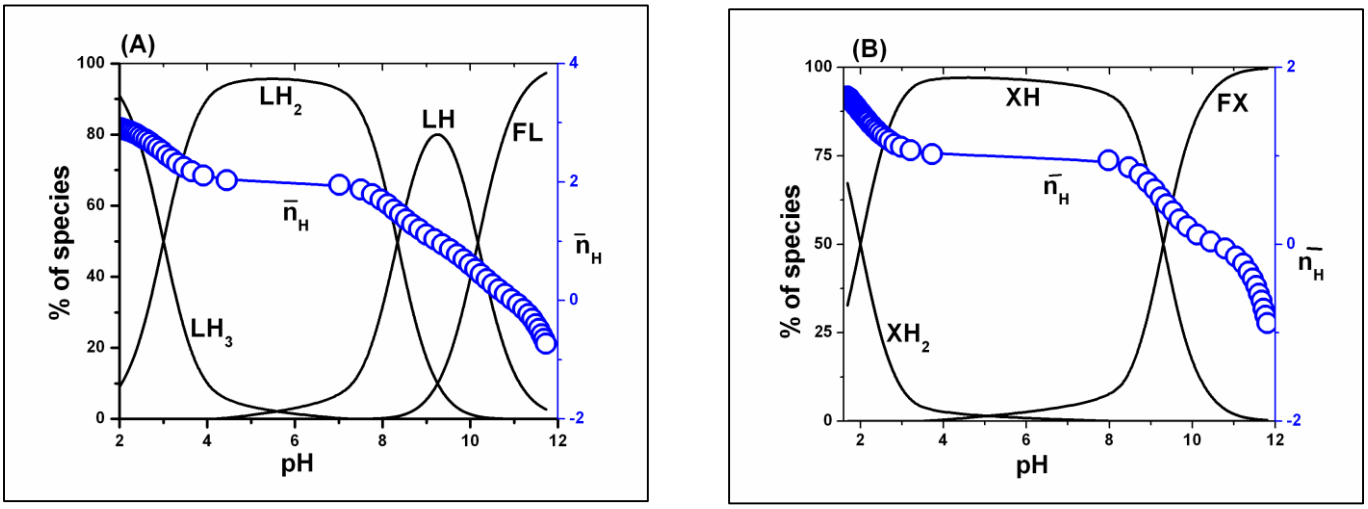


Fig. 3: Formation functions (○) and species distribution diagrams of (A) Cysteine and (B) Methionine in aqueous medium. Amount of Cyst and Met are 0.375 mmol

*Table 2: Effect of systematic errors in influential parameters on the protonation constants of Cysteine and Methionine in 50% v/v 1, 4-Dioxan-water mixtures*

| *Ingredient* | *% Error* | *L- Cysteine* | | |
|---|---|---|---|---|
| | | *log β1(SD)* | *logβ2(SD)* | *logβ3(SD)* |
| | *0* | *10.72(02)* | *18.98(02)* | *21.84(03)* |
| *Alkali* | *-5* | *20.40(**)* | *30.73(**)* | *34.80(**)* |
| | *-2* | *10.84(02)* | *19.14(02)* | *21.80(03)* |
| | *+2* | *10.47(02)* | *18.44(03)* | *20.93(04)* |
| | *+5* | *10.21(03)* | *17.92(05)* | *20.28(06)* |
| *Acid* | *-5* | *10.34(03)* | *18.11(05)* | *20.37(06)* |
| | *-2* | *10.52(02)* | *18.51(03)* | *20.96(04)* |
| | *+2* | *10.77(02)* | *19.04(02)* | *21.74(03)* |
| | *+5* | *Rejected* | *Rejected* | *Rejected* |
| *Ligand* | *-5* | *8.29(**)* | *16.27(**)* | *19.23(**)* |
| | *-2* | *10.59(02)* | *18.69(02)* | *21.28(03)* |
| | *+2* | *10.71(02)* | *18.86(02)* | *21.41(03)* |
| | *+5* | *10.79(02)* | *18.98(02)* | *21.71(03)* |
| *log F* | *-5* | *10.63(02)* | *18.74(02)* | *21.26(03)* |
| | *-2* | *10.64(02)* | *18.76(02)* | *21.31(03)* |
| | *+2* | *10.59(02)* | *18.79(02)* | *21.39(03)* |
| | *+5* | *10.67(02)* | *18.81(02)* | *21.44(03)* |
| | | *L- Methionine* | | |
| | *0* | *8.87(01)* | *11.95(02)* | -- |
| *Alkali* | *-5* | *9.48(**)* | *13.02(**)* | -- |
| | *-2* | *8.77(01)* | *11.63(02)* | -- |
| | *+2* | *8.48(03)* | *11.15(04)* | -- |
| | *+5* | *8.24(04)* | *10.78(06)* | -- |
| *Acid* | *-5* | *8.46(03)* | *11.00(04)* | -- |
| | *-2* | *8.64(02)* | *11.39(03)* | -- |
| | *+2* | *8.89(01)* | *11.89(01)* | -- |
| | *+5* | *9.06(02)* | *12.29(02)* | -- |
| *Ligand* | *-5* | *7.65(**)* | *11.17(**)* | -- |
| | *-2* | *8.62(02)* | *11.39(03)* | -- |
| | *+2* | *8.67(02)* | *11.39(03)* | -- |
| | *+5* | *8.70(02)* | *11.39(03)* | -- |
| *log F* | *-5* | *8.76(01)* | *11.58(02)* | -- |
| | *-2* | *9.08(16)* | *12.76(22)* | -- |
| | *+2* | *8.78(01)* | *11.66(02)* | -- |
| | *+5* | *8.79(01)* | *11.70(02)* | -- |

*Standard deviation very high*

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 behavior 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

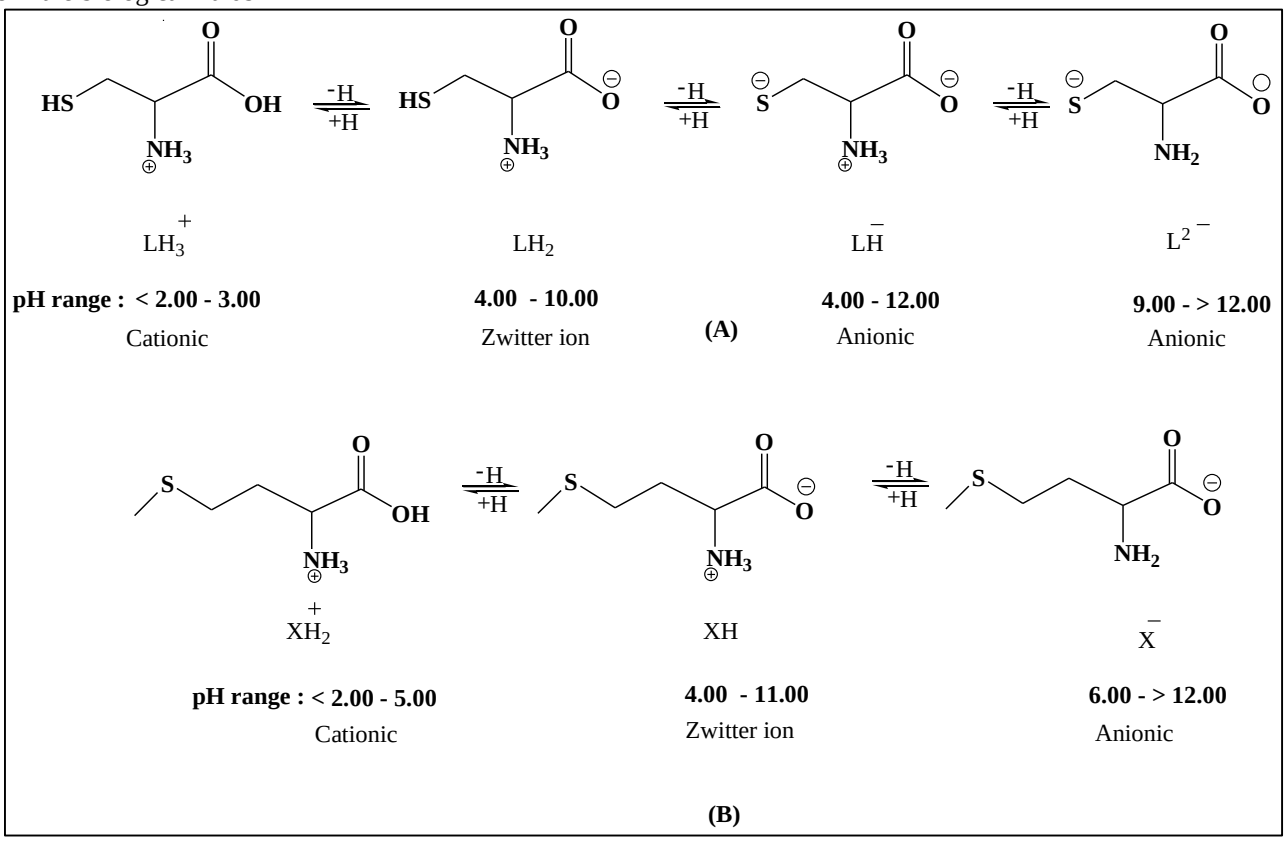


Fig. 4: Protonation-deprotonation equilibria of (A) Cysteine and (B) Methionine

## IV. CONCLUSIONS

1) Cysteine and Methionine forms $LH_3^+/XH_2^+$ at low pH and gets deprotonated with the formation of $LH_2$, $LH^-$ and $L^{2-}/XH$, and $X^-$ Successively with increase in pH.
2) The log K values of protonation constants increase linearly with decreasing dielectric constant of DOX- water mixtures. This trend indicates the dominance of electrostatic forces over than 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.

## ACKNOWLEDGEMENTS

The author thanks the University Grants Commission, Government of India, New Delhi, for financial support under Minor Research Project.

## REFERENCES

[1] C. Jacob, I. Knight & P. G. Winyard "Aspects of the Biological Redox Chemistry of Cysteine: from Simple Redox Responses to Sophisticated Signaling Pathways" Biol. Chem., 387: p1385-1397, 2006.
[2] B. Balta, G. Monard, M. F. Ruiz-Lopez, M. Antoine, A. Gand, S. Boschi-Muller & G. Branlant "Theoretical Study of the Reduction Mechanism of Sulfoxides by Thiols" J. Phys. Chem. A, 110: p7628-7636, 2006.
[3] D. W. Martin, P. A. Mayes, V. W. Food Wells & D. W. Granner "Harper's Review of Biochemistry" 20th Ed., Lange Medical Publishers, California, 672, 1985.
[4] B. Regland, L. Abrahamsson, K. Blennow, B. Grenfeldt & C. G. Gotlfries "Cerebrospinal fluid-methionine is elevated in psychotic patients" J. Neural Transm, 111, p631–640, 2004.
[5] M. Noji & K. Saito "Sulfur amino acids: Biosynthesis of Cysteine and Methionine, Sulphur in plants" A review, p135-144, 2003.
[6] G.N. Rao, & R.S. Rao, "Characteristics of water-organic solvent mixtures" J. Teach. Res. Chem., 2: p15-27, 1995.
[7] H. Sigel, R.B. Martin, R. Tribolet, U. K. Haring & R. M. Balakrishnan, "An estimation of the equivalent solution dielectric constant in the active-site cavity of metalloenzymes dependence of carboxylate metal ion complex stabilities on the polarity of mixed aqueous/organic solvents" Eur. J. Biochem. 152: p187-194, 1985.
[8] S.Herbert Harned & M. Clair Birdsall, '' The Acidic Ionization Constant of Glycine in Dioxane-Water Solutions'' J. Am. Chem. Soc. 65: p54-57, 1943,

[9] A. Sharma, K. D. Gupta & K. K Sexsena, ''The stability constants of Cu (II) complexes''J Indian Council Chem., 19: p43-50, 2002.
[10] V. S. Rao, P. S. Rao, B. Srikanth, C. Kamala Sastry & G. N. Rao, ''Speciation of binary complexes of Co(II), Ni(II), Cu(II) and Zn(II) with L-aspartic acid in anionic, cationic and neutral micellar medium'' Chem. Speciat. Bioavail. 21: p71-80, 2009.
[11] J. Hens, ''Structural effects on equilibria in organic chemistry'' Wiley, New York, 1975.
[12] S. H. Herbert & M. Clair Birdsall, ''The Acidic Ionization Constant of Glycine in Dioxane-Water Solutions'' J. Am. Chem. Soc., 65: p54–57, 1943.
[13] G. Gran, "Determination of the equivalence points in potentiometric titrations-Part-II" Analyst, 77: p661-671, 1952.
[14] G. Gran, "Equivalence volumes in potentiometric titrations" Anal. Chim. Acta, 206: p111-123, 1988.
[15] K. V. Santhe Devi, B. Rama Raju & G.N. Rao, "Efect of Dielectric Constant on protonation Equlibria of L-Dopa and 1, 10-Phenanthroline in Dioxan-Water Mixtures" Acta Chim. Slov., 57: p398-404, 2010.
[16] M. P. Latha, V. M. Rao, T.S Rao & G.N. Rao, "Determination of protonation constants of L-glutamic acid and L-methionine in 1, 2-propanediol-water mixtures" Acta Chim. Slov, 54: p160-165, 2007.
[17] G. N. Rao, (1989) "Complex equilibria of some biologically important metal ions in aquo-organic media" Ph. D. Thesis. Andhra University, Visakhapatnam, India.
[18] P. Gans, A. Sabatini & A. Vacca, "An improved computer program for the computation of formation constants from potentiometric data" Inorg. Chim. Acta, 18: p237-239, 1976.
[19] M. Born, "Volumes and hydratation-swarme der ionen" Z. Phys., 1: p45-48, 1920.