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Protonation Equilibria of L-Methionine and L-Cysteine In Cationic Micellar Media

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ABSTRACT

Knowledge of the protonation constants of amino acids is important and necessary for complete understanding of their physicochemical behaviour. Protonation equilibria of L-methionine and L-cysteine in varying compositions (0.0–2.5 % w/v) of CTAB – water mixtures were investigated pH-metrically. Titrations were performed at 303.0 K and the ionic strength of the medium was maintained at 0.16 mol.L^{-1} using sodium chloride. The protonation constants have been calculated with the computer program MINIQUAD 75 and are selected based on statistical parameters. The best fit chemical models of the protonation equilibria were based on crystallographic R-factor, skewness and kurtosis. Linear and non-linear variations of stepwise protonation constants with reciprocal of dielectric constant of the solvent mixtures have been attributed to the dominance of electrostatic and non-electrostatic forces respectively. Distribution of species, protonation equilibria and effect of influential parameters on the protonation constants has also been presented.

KEYWORDS: Protonation equilibria, L-methionine, L-cysteine, CTAB, MINIQUAD75

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INTRODUCTION

A number of studies have been reported on protonation constants of α-amino acids in different media [1-3]. Acidity and basicity of a molecule is governed by its structure and solvent effects [4]. A review of literature has revealed that a little is reported for protonation constants of of L-Methionine and L-Cysteine in low dielectric media. The present study reveals the determination of protonation constants of L- Methionine and L-Cysteine in CTAB-water mixtures.

Methionine (Met) is an important essential amino acid in humans[5]. It plays a critical role in the metabolism and health of many species including humans. Supplementation of Met benefits the patients suffering from copper poisoning. It is one of two sulfur-containing proteinogenic amino acids, an intermediate in the biosynthesis of cysteine, carnitine, taurine, lecithin, phosphatidylcholine and other phospholipids. All plants and some forms of bacteria can synthesize Met by two different sequences[6]. Mammalian tissues and many bacteria synthesize it from cobalamine enzyme where as all the plants and some bacteria synthesize it from homocysteine.

Cysteine (Cys)[7] is a semi-essential proteinogenic amino acid. The thiol side chain in Cys often participates in enzymatic reactions as a nucleophile, susceptible to oxidation to give the disulfide derivative cystine, which serves an important structural role in many proteins. It is catabolised in the gastrointestinal tract and blood plasma. It is important for protein synthesis, detoxification and diverse metabolic functions. It is found in beta-keratin, the main protein in nails, skin and hair. Its therapy has proved excellent for treatment of asthmatics enabling them to stop theophylline and other medications. The role and importance of sulphur containing amino acids as well as redox chemistry of Cys residues in enzymes is summarized[8,9].

Cetyl trimethyl ammonium bromide [CTAB] is a quaternary ammonium cationic surfactant. It is one of the components of the topical antiseptic cetrimide[10]. It profoundly influences the bulk properties of physiological systems. They can solubilise, concentrate and compartmentalize ions and molecules[11]. It is also one of the main components of some buffers for the extraction of DNA[12]. It has emerged as the preferred choice for biological use because it maintains the integrity of precipitated DNA during isolation[13]. It has been shown to have potential use as an apoptosis-promoting anticancer agent for head and neck cancer (HNC)[14]. Surfactants play a key role in nanoparticle synthesis by adsorbing to the surface of the forming nanoparticle and lowering its surface energy[15].

MATERIALS AND METHOD

Chemicals and standard solutions

All the chemicals used in this investigation were of analytical reagent grade purity. 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 CTAB(Hi-media) were prepared. Sodium chloride(Merck, India) of 2.0 M was prepared to maintain the ionic strength in the titrand. Triple-distilled deionised water was used for preparation of all the solutions. The acid and base solutions were standardised by standard methods. To assess the errors that might have crept into the determination of the concentrations, the data were subjected to analysis of variance of one way classification (ANOVA)[16]. The strengths of alkali and mineral acid were determined using the Gran plot method[17,18].

Alkali metric titrationss

Alkalimetric titrations were carried out in media containing varying compositions of CTAB-water (0.5–2.5% w/v) maintaining an ionic strength of 0.16 mol L^{-1} with sodium chloride at 303.00 ± 0.05 K. An Elico LI-120 pH meter was used. Potassium hydrogen phthalate(0.05 mol L-1) and borax (0.01 mol L-1) 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 ligands in the titrands ranged between 0.25 and 0.50 mmol. The glass electrode was equilibrated in a well stirred CTAB-water mixture containing inert electrolyte for several days. At regular intervals strong acid was titrated against alkali to check the complete equilibration of the glass electrode. The calomel electrode was refilled with CTAB-water mixture of equivalent composition as that of the titrand. The details of experimental procedure and titration assembly have been discussed elsewhere [19].

Modelling strategy

The approximate protonation constants of Met and Cys were calculated with the computer program SCPHD(Rao,1989).The best fit chemical model for each system investigated was arrived at using non-linear least squares computer program, MINIQUAD75 (Gans *et al.*,1976), which exploits the advantage of constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm. The variation of stepwise protonation constants (log K) with the mole fraction of the medium was analysed on electrostatic grounds for the solute–solute and solute–solvent interactions.

RESULTS AND DISCUSSION

The best fit models containing the type of species and overall formation constants along with some of the important statistical parameters are given in Table 1. A very low standard deviation (SD) in log β values indicates the precision of these parameters. The small values of U_{corr} (sum of squares of deviations in concentrations of ligand and hydrogen ion at all experimental points) indicate that the experimental data can be represented by the model. The small values of the mean, standard deviation and the mean deviation for the systems corroborate that the residuals are around zero mean with little dispersion. The kurtosis values in the present study indicate that the residuals form

leptokurtic pattern in the case of Met and Cys. The values of kurtosis given are between 2.04 and 19.48 and skewness recorded are between –0.01 and 0.42. These data evince that the residuals form part of the normal distribution hence, the least squares method can be applied to the present data. The sufficiency of the model is further evident from the low crystallographic R-values. These statistical parameters thus show that the best fit models portray the acid–base equilibria of Met and Cys in CTAB-water mixtures. Alkali metric titration data are simulated using the model parameters given in Table 1. These data are compared with the experimental alkali metric titration data, to verify the sufficiency of the models. The overlap of the typical experimental and simulated titrations data given in Figure 1 indicates that the proposed models represent the experimental data.

Table1: Best fit chemical models of protonation equilibrium of L-methionine and L-cysteine in CTAB-water mixtures Temp= 303K, Ionic strength=0.16moldm^{-3}.

w/v CTAB	log β1(SD)	log β2(SD)	log β3(SD)	NP	Ucorrx108	Skegness	Kurtosis	χ2	R-factor
	MET (pH range 1.80-9.80)								
0	8.93(07)	11.17(09)	---	54	26.23	0.42	5.45	5.93	0.0410
0.5	9.44(04)	11.42(05)	---	80	30.29	-0.24	2.27	6.50	0.0311
1.0	9.29(02)	11.94(04)	---	110	20.19	-2.72	19.48	32.15	0.0233
1.5	9.43(02)	11.85(03)	---	90	14.94	0.07	2.78	5.96	0.0200
2.0	9.15(02)	11.35(03)	---	84	12.44	-0.01	3.15	12.76	0.0199
2.5	9.51(03)	11.83(03)	---	91	15.03	-0.33	2.85	12.90	0.0211
	CYSTEINE (pH range 2.20-10.50)								
0	10.53(02)	18.67(01)	20.53(09)	60	14.13	-0.83	6.41	22.67	0.0241
0.5	10.88(02)	18.77(01)	20.62(05)	72	49.25	-0.09	2.64	8.11	0.0403
1.0	10.40(04)	18.20(04)	20.27(08)	79	60.30	-0.80	2.04	51.92	0.0473
1.5	10.78(03)	19.04(02)	20.66(09)	42	14.08	-0.22	2.71	15.43	0.0254
2.0	10.44(02)	20.56(02)	20.56(04)	75	24.16	-0.57	3.26	18.96	0.0285
2.5	10.35(03)	20.04(02)	20.04(01)	50	27.15	-0.76	2.45	36.19	0.0335

U_{corr}=U/(NP-m), where m=number of species; NP=Number of experimental points; SD=standard deviation

Secondary formation functions

Secondary formation functions such as the average number of protons bound per mole of ligand $\bar{n}_H$ and the number of moles of alkali consumed per mole of ligand (**a**) are useful for detecting the number of equilibria. Plots of $\bar{n}_H$ versus pH for different concentrations of the ligand should overlap, if there is no formation of polymeric species. Overlapping formation curves for Met and Cys (Figure 1) rule out the polymerisation of the ligand molecules. The pH values at half integral values of $\bar{n}_H$ correspond to the protonation constants of the ligands. Two half integrals (1.5 and 0.5) in the case of L- Met (Figure 1A) emphasise the presence of two protonation deprotonating equilibria in the pH range of present study. Three half integrals (2.5, 1.5 and 0.5) in the case of Cys (Figure 1B) emphasise the presence of three protonation deprotonating equilibria in the pH range of present study. The number of plateaus in the formation curves corresponds to the number of these equilibria. The plots of **a** versus pH are given in Figure 2. The negative values of **a** correspond to the number of moles of free acid present in the titrand and the number of associable protons. The positive values of

a indicate the number of dissociable protons in the ligand molecules. The maximum value of **a** in Figure 2(A) is +1, which indicates that L- Met has one dissociable (one carboxyl) proton. The corresponding value for Cys Figure 2(B) is 2, which clearly infers that Cys has two dissociable protons (one carboxyl) proton and one from HS proton

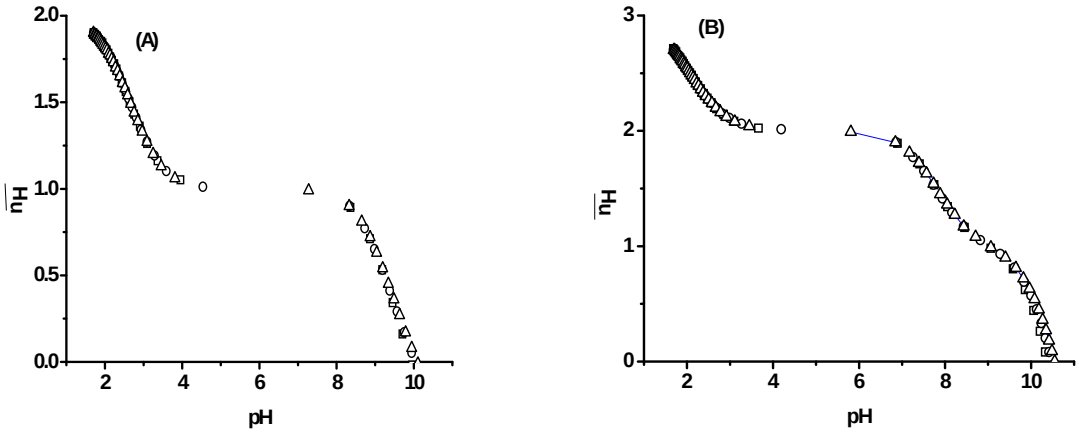


Fig 1: Plots of $\bar{n}_H$ versus pH of (A) Met and (B) Cys: (0.25, (○) 0.375, and (Δ) 0.50 mmol, respectively in 1.0% w/v CTAB- water mixture.

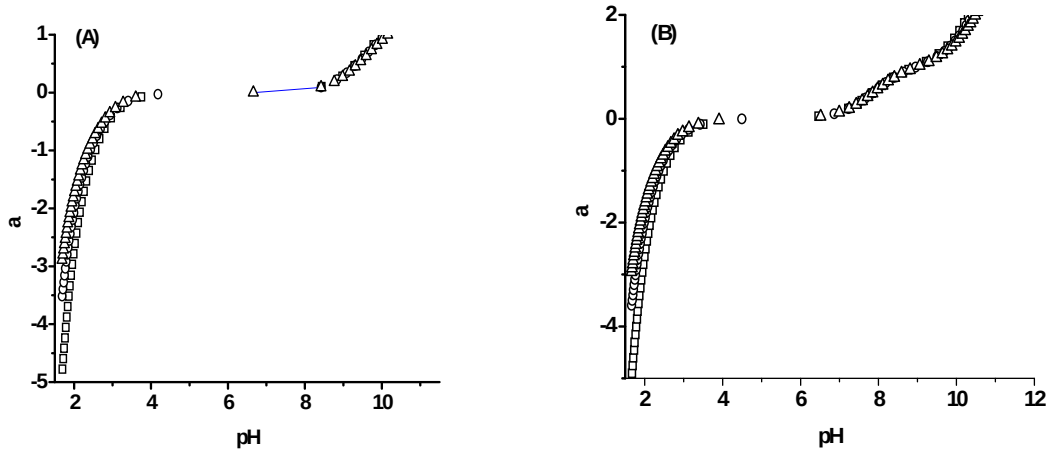


Fig 2: Variation of a with pH in 1.5 % w/v CTAB–water mixture; of (A) Met and (B) Cys0.25, (○) 0.375, and (Δ) 0.50 mmol, respectively in 1.0% w/v CTAB- water mixture.

Distribution diagrams

Typical distribution plots produced by DISPLOT (Rao *et al.*, 1989) using protonation constants from the best fit models are shown in Figure 3. A single representative plot is shown for each system at a particular CTAB-water concentration. The zwitter ions of Met LH is present to an extent of 98% in the pH range 1.5-11.0. The distribution plot of Met in Figure 3(A) shows the existence of LH_2, LH, FL. In the case of Cys LH^- is present to an extent of 98.5% in the pH range 1.5-9.0. The distribution plot of Cys in Figure 3(B) shows the existence of LH_2, L^{2-}. The corresponding protonation–deprotonation equilibrium are shown in Figure 3.

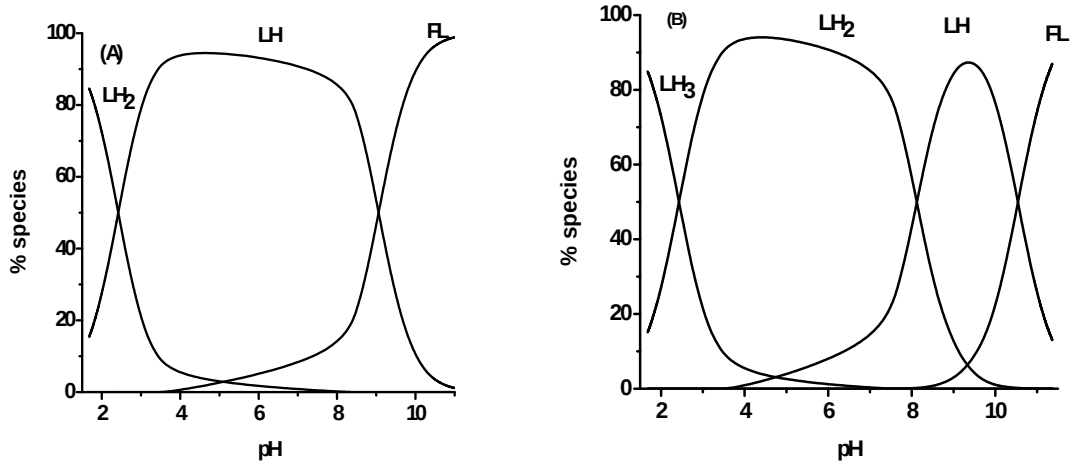


Fig 3: Species distribution diagrams of (A) Met and (B) Cys in 1.0% w/v CTAB-water mixture

Effect of surfactant

The above results show that the shifts in prolongation constant values are more in the case of cationic surfactant CTAB. Both the acids behave more strongly in CTAB whose interfacial layer is positively charged. This is mainly because the anion from acid molecule, $X(z-1)$ is strongly attracted to the interfacial layer of the cationic surfactants due to attraction between opposite charges. This creates a strain on the acid molecule and hence the acid dissociates more strongly. Many workers were of the opinion that both electrostatic and non-electrostatic effects should be considered even in the case of simple acid–base equilibria, one dominates the other depending upon the nature of solute and solvent (Schneider *et al.*, 1976; Feakins *et al.*, 1983). Born's classical treatment (Born, 1920) holds good in accounting for the electrostatic contribution to the free energy change. The number of micelles increases with the concentration of surfactant and oppositely charged ions and are concentrated in the Stern layer. According to Born's classical treatment the energy of electrostatic interaction or the logarithm of step-wise protanation constant (log K) should vary linearly as a function of mole fraction of the medium. Such linear or almost variation of the protonation constants of L- Met [Figure 4(A)] in CTAB–water mixture shows the dominance of non-electrostatic interactions. In the case of Cys [Figure 4(B)] in the CTAB–water mixture shows the dominance of non-electrostatic interactions. Micelles alter the reaction rates and shift equilibria primarily by concentrating reactants within the small volume of micellar pseudo phase. The extent of change is a product of the micellar effect on the reaction within the micellar pseudo phase and the distribution of reactants between the two phases. The effect of micelles on overall reaction rates and equilibria depends upon the incorporation of solutes into the micellar pseudo phase.

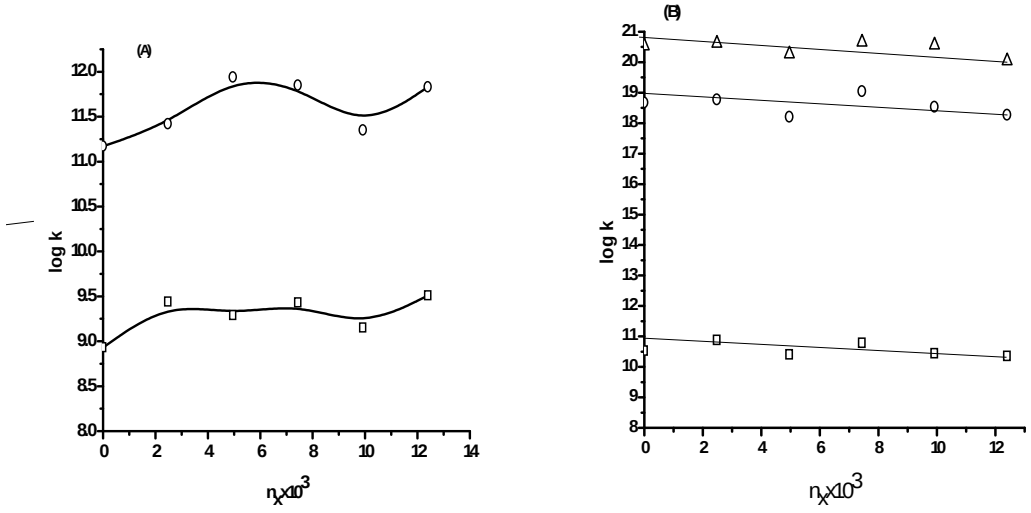


Figure 4: Variation of stepwise prolongation constant (log K) with mole fraction of CTAB in CTAB–water mixtures. (A) L- Met □) logK1, (○) logK2, (B) Cys (□) logK1, (○) logK2, (Δ) log k3

Effect of systematic errors in best fit model

MINIQUAD75 does not have the ability to study the effect of systematic errors in the influential parameters such as the concentration of ingredients and electrode calibration on the magnitude of the protonation constant. In order to rely upon the best chemical model for critical evaluation and application under varied experimental conditions with different experimental and accuracies of data acquisition an investigation was undertaken that introduced pessimistic errors into the concentration of alkali, mineral acids and the ligands. The results of a typical system given in Table 2 emphasise that the errors in the concentrations of alkali and mineral acid affects the protonation constants more than that of the ligand.

Table 2: Effect of systematic errors in influential parameters on the protonation constants of Met and Cys in 0.5% w/v CTAB-water mixture

Ingredient	% Error	log β_{mlh} (SD)				
		Met		Cys		
		11	12	11	12	13
	0	9.44(04)	11.42(05)	10.88(02)	18.77(01)	20.62(05)
Alkali	-5	10.41(63)	Rejected	11.65(43)	19.45(25)	Rejected
	-2	9.75(52)	11.68(22)	12.23(62)	18.95(20)	21.85(32)
	+2	Rejected	11.56(18)	11.10(15)	Rejected	20.92(17)
	+5	10.22(25)	11.55(32)	Rejected	19.20(16)	21.56(34)
Acid	-5	9.65(23)	11.53(24)	11.32(28)	18.79(10)	20.97(18)
	-2	9.54(18)	11.49(26)	Rejected	18.45(12)	20.98(16)
	+2	9.55(26)	11.11(32)	10.34(23)	18.61(14)	Rejected
	+5	10.14(22)	11.22(22)	10.94(25)	18.56(18)	20.48(18)
Ligand	-5	9.34(23)	11.32(12)	11.05(15)	18.92(21)	20.76(20)
	-2	9.64(34)	11.48(18)	11.45(23)	18.65(16)	20.44(30)
	+2	9.34(13)	11.22(19)	10.97(23)	18.88(14)	20.34(19)
	+5	9.58(15)	11.34(24)	10.96(25)	18.86(29)	20.59(18)

A. Residual Analysis

In data analysis with least squares methods, the residuals (the differences between the experimental data and the data simulated based on the model parameters) are assumed to follow Gaussian or normal distribution. 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 residuals form leptokurtic patterns. The values of skewness given in Table 1 are between -0.01 and 0.42. These data evince that the residuals form 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. These statistical parameters thus show that the best fit models portray the acido-basic equilibria of Met and Cys in CTAB water mixtures.

B. $\chi 2$ test

$\chi 2$ is a special case of gamma distribution whose probability density function is an asymmetrical function. This distribution measures the probability of residuals forming a part of standard normal distribution with zero mean and unit standard deviation. If the $\chi 2$ calculated is less than the table value, the model is accepted.

C. Crystallographic R-Test

Hamilton's R factor ratio test is applied in complex equilibria to decide whether inclusion of more species in the model is necessary or not. In pH metric method the readability of pH meter is taken as the R limit which represents the upper boundary of R beyond which the model bears no significance. When these are different numbers of species the models whose values are greater than R-table are rejected. The low crystallographic R-values given in Table 2 indicate the sufficiency of the model.

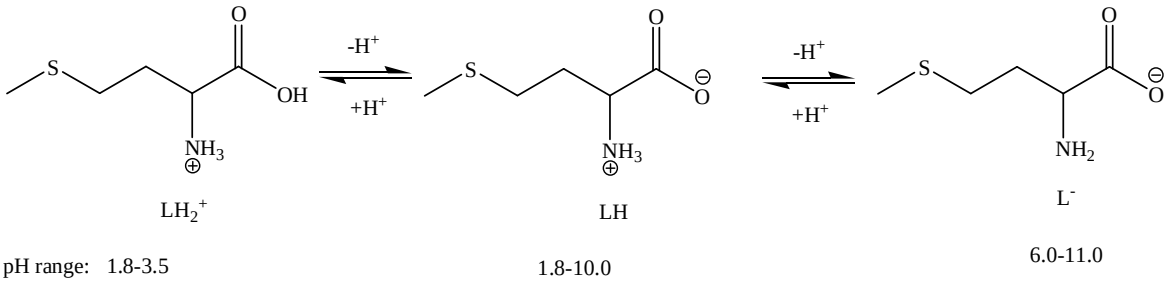
D. Skewness

It is a dimensionless quantity indicating the shape of the error distribution profile. A value of zero for skewness indicates that the underlying distribution is symmetrical. If the skewness is greater than zero, the peak of the error distribution curve is to the left of the mean and the peak is to the right of the mean if skewness is less than zero. The values of skewness recorded in Table 1 are between -0.01 and 0.42. These data evince that the residuals form a part of normal distribution hence, least-squares method can be applied to the present data.

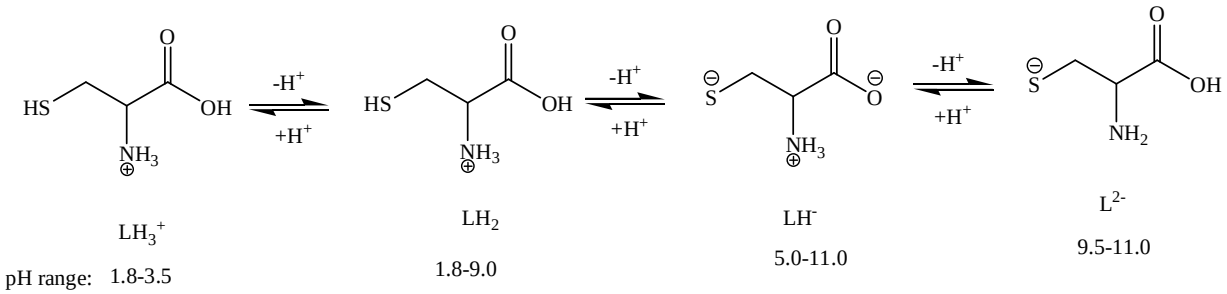
E. Kurtosis

It is a measure of the peakedness of the error distribution near a model value. For an ideal normal distribution kurtosis value should be three (mesokurtic). If the calculated kurtosis is less than three, the peak of the error distribution curve is flat (platykurtic) and if the kurtosis is greater than

three, the distribution shall have sharp peak (leptokurtic). The kurtosis values in the present study indicate that the residuals form leptokurtic pattern in the case of Met and platykurtic for Cys.



Protonation-deprotonation equilibria of L-methionine



Protonation-deprotonation equilibria of L-cysteine

CONCULSIONS

1. Met has one dissociable proton and one amino group which can associate with a proton. It exists as LH_2^+ at lower pH and deprotonates as the pH increases and forms LH and L^- respectively.
2. Cys has two dissociable protons and one amino group which can associate with a proton. It exists as LH_3^+ at lower pH and deprotonates as the pH increases and forms LH_2^+, LH and L^- respectively.
3. The linear variation of log k values varies non-linearly for Met and linearly for Cys.
4. The linear variation is due to the dominance of electrostatic forces in the protonation-deprotonation equilibria. The non-linear variation of Cys indicates the dominance of non-electrostatic interactions between solute and solvent.
5. The effect of systematic errors in the influential parameters shows that the errors in the concentrations of alkali and mineral acids will affect the protonation constants more than that of the ligand.

REFERENCES

1. S. Herbert Harned & M. Clair Birdsall, □ The Acidic Ionization Constant of Glycine in Dioxane-Water Solutions" J. Am. Chem. Soc., 1943;65: 54-57.

2. A. Sharma, K. D. Gupta & K. K Sexsena, □The stability constants of Cu (II) Complexes''J Indian Council Chem., 2002; 19: 43-50.
3. 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 neutral micellar medium'' Chem. Speciat. Bioavail., 2009; 21: 71-80.
4. J. Hens, □Structural effects on equilibria in Organic Chemistry'' Wiley, New York, 1975.
5. "Nomenclature and Symbolism for Amino Acids and Peptides". IUPAC-IUB Joint Commission on Biochemical Nomenclature, 1983. Archived from the original on 9 October 2008. Retrieved 5 March. 2018.
6. D. W. Martin, P. A. Mayes, V. W. Food Wells, D. W. Granner, Harper's Review of Biochemistry, 20th Ed., Lange Medical Publishers, California 1985; 672.
7. "Nomenclature and symbolism for amino acids and peptides (IUPAC-IU Recommendations 1983)" Pure Appl. Chem., 1984; 56(5): 595–624.
8. C. Jacob, I. Knight & P. G. Winyard "Aspects of the Biological Redox Chemistry of Cys from Simple Redox Responses to Sophisticated Signalling Pathways" Biol. Chem., 2006; 387: 1385-1397.
9. 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, 2006; 110: 7628-7636
10. Laemmli, U. K. "Cleavage of structural proteins during the assembly of the head of bacteriophage T4". Nature. 15 Aug. 1970; 227 (5259): 680–685.
11. E. Pelizetti and E. Pramaro, Anal. Chim. Acta 1983;1: 169.
12. Clarke, Joseph D. "Cetyltrimethyl Ammonium Bromide (CTAB) DNA Miniprep for for Plant DNA Isolation" Cold Spring Harbor Protocols, 01 Mar. 2009(3).
13. MA Azmat, IA Khan, HM Cheema, Rajwana, IA; Khan, AS; Khan, AA ("Extraction of DNA suitable for PCR applications from mature leaves of Mangifera Indica L" 2012.
14. Ito, Emma, Yip, Kenneth W, Katz, Dad, Fonseca, Sonali B. Hedley, David W.; Chow, Sue; Xu, G. Wei; Wood, Tabitha E.; Bastianutto, Carlo "Potential Use of Cetrimonium Bromide as an Apoptosis-Promoting Anticancer Agent Head and Neck Cancer". Molecular Pharmacology. 01 Nov. 2009;76(5): 969–983.
15. S. K. Mehta, Kumar, Sanjay, Chaudhary, Savita, Bhasin, K. K. "Effect of Cationic Surfactant Head Groups on Synthesis, Growth and Agglomeration Behavior of Nano particles". Nanoscale Research Letters. 01 July 2009;4(10): 1197–1208.

16. R. S. Rao and G. N. Rao, Computer Applications in Chemistry, Himalaya Publishing House, Mumbai 2005; 302.
17. Gran G. Determination of the equivalence point in potentiometric titrations. Analyst. 1952; 77: 661.
18. Gran G. Equivalence volumes in potentiometric titrations. Anal Chim Acta 1988;206:111.
19. Latha M. P, Rao V. M, Rao T. S, Rao G. N., Bull. Chem. Soc. Ethiop 2007; 21: 363.