

PROJECT WORK FOR THE ACADEMIC YEAR 2018-2019

PROTONATION EQUILIBRIA OF SULFUR CONTAIN AMINO ACIDS  
OF L-METHIONINE AND L-CYSTEINE IN AQUEOUS MEDIUM



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Regd. No.

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## Certificate

Certified that this is bonafide Record of Practical  
work done by Mr. / Humari M. Kiran Kumar

of III BSc MPC E.M Class in Chemistry Laboratories  
of D. S. Krishna Govt Degree College during the years

2018 - 2019

No. of Experiments done and certified \_\_\_\_\_

  
Lecturer in-Charge

Date

*Verified  
11/13*

  
Head of the Department

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## INTRODUCTION

### Amino acid metabolism

The properties common in all the amino acids are due to carboxyl and amino groups and their specific arrangement pattern. In aqueous solution at neutral pH, amino acids exist as zwitterions. They are capable of acting both as weak acids and weak bases because either the amine group is protonated to form  $\text{-NH}_3^+$  at low pH or the carboxylic acid group is deprotonated to form  $\text{-CO}_2^-$  at high pH.

Each amino acid has at least a minimum of two characteristic dissociation constants which are normally expressed in terms of pK values. The solubility of each amino acid is minimal at its isoelectric pH. The approximate pK values for  $\alpha$ -carboxyl and  $\alpha$ -amino groups of  $\alpha$ -amino acid are 2 and 10, respectively. If there are more dissociating groups in the amino acid molecule, then it will have more pK values. Knowledge of these pK values is useful in the separation and isolation of each amino acid by ion-exchange chromatography.

All amino acids are derived from intermediates in glycolysis, the citric acid cycle, or the pentose phosphate pathway (Fig.1). Nitrogen enters these pathways by way of glutamate and glutamine. Organisms vary greatly in their ability to synthesize the 20 common amino acids. Whereas most bacteria and plants can synthesize all 20, mammals can synthesize only about half of them. These are the non-essential amino

acids, not needed in the diet (Table 1). Non-essential amino acids can be synthesized in the human or animal bodies from other cellular metabolites when needed.

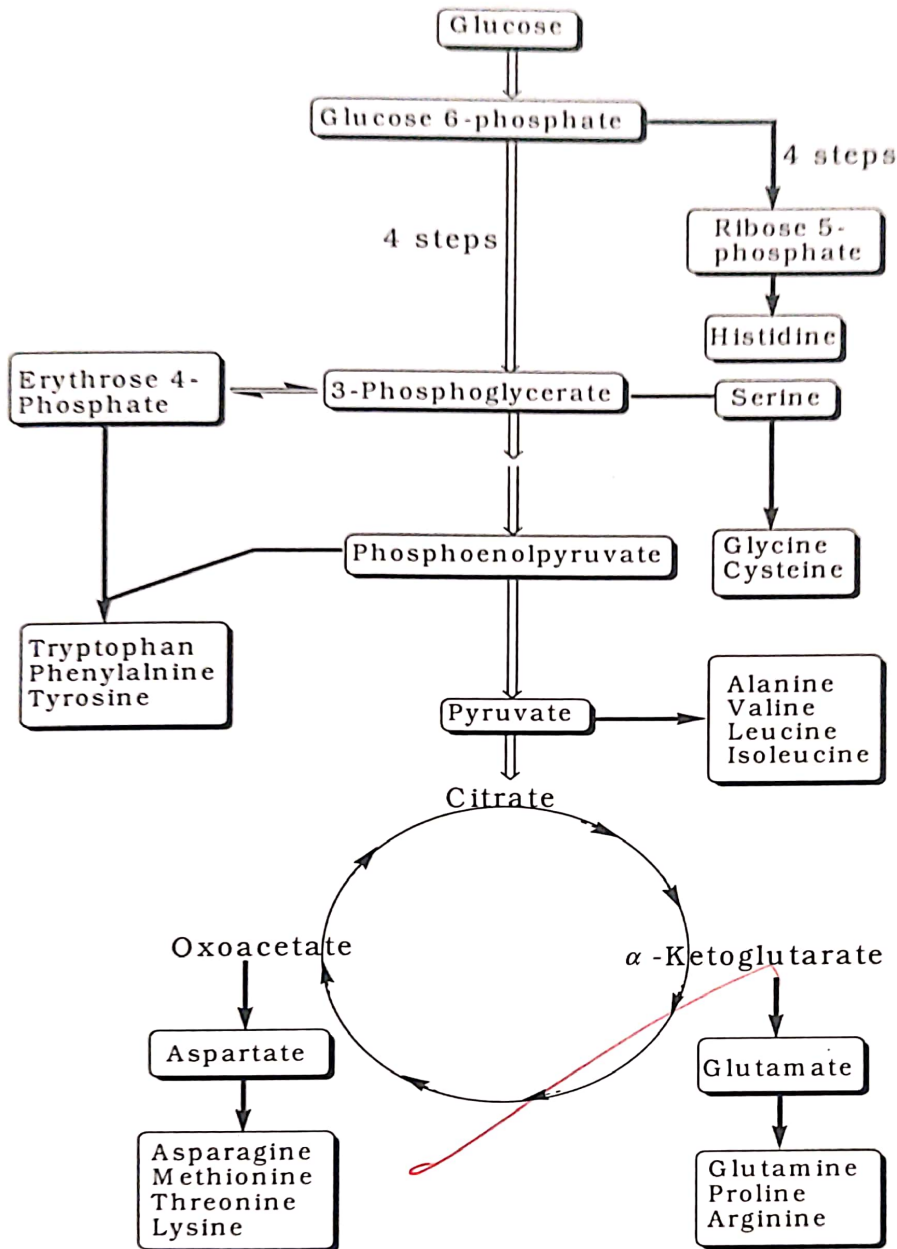
**Table 1:** Non-essential and Essential Amino Acids for Humans

<b>Nonessential</b>	<b>Conditionally essential</b>	<b>Essential</b>
Alanine(Ala) <b>Asparagine(Asn)</b> <b>Aspartate(Asp)</b> <b>Glutamate(Glu)</b> <b>Serine</b>	<b>Arginine(Arg)</b> <b>Cysteine(Cys)</b> <b>Glutamine(Gla)</b> <b>Glycine(Gly)</b> <b>Proline(Pro)</b> <b>Tyrosine(Tyr)</b>	<b>Histidine(His)</b> <b>Isoleucine</b> <b>Leucine</b> <b>Lysine</b> <b>Methionine(Met)</b> <b>Phenylalanine</b> <b>Threonine</b> <b>Tryptophan</b> <b>Valine(Val)</b>

The essential amino acids must be obtained from food. Plants or other biological systems can synthesize amino acids from simple inorganic compounds, but animals rely on adequate supplies in their diet.

The non-availability of essential amino acids leads to an overall deficiency of the protein from the body resulting in weight loss, muscle weakness and anemia. Specific disorders associated with deficiencies of particular amino acids are given below.

- Arthritis** : **Histidine**
- Insomnia** : **GABA**
- Allergies** : **Methionine**
- Depression** : **S-Adenosyl-Methionine (SAME)**
- Heart disease** : **Carnitine**
- Gastrointestinal problems** : **Glutamine**



**Fig. 1:** Overview of amino acid biosynthesis.

### **Biological Importance**

Methionine deficiency is rare, but when present it may lead to reduced growth rate along with liver damage, muscle loss, edema, skin lesions and lethargy. High levels of Methionine can be found in eggs.

sesame seeds, Brazil nuts, fish, meats and some other plant seeds; Methionine is also found in cereal grains. Most fruits and vegetables contain very little of it. Most legumes are also low in Methionine.

Methionine is a powerful antioxidant, and the sulfur it contains helps neutralize free radicals that are formed as a result of various metabolic processes in the body. If not neutralized, free radicals interact with DNA and the proteins in healthy cells and damage tissues and organs such as skin, nails, heart, liver and lungs.

Methionine also plays an important role in the proper functioning of the immune system. High Methionine levels in the body increase the levels of other amino acids such as glutathione, homocysteine and taurine. But, while glutathione and taurine help improve immune function and suppress inflammatory reactions, increased homocysteine levels may have the opposite effect.

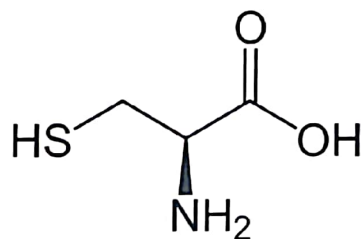
### ***Toxicity***

Certain nutritional and metabolic studies have employed amounts of Methionine, including the D and DL isomers, both below and above the requirement and have not reported adverse effects in adults and children. Although Methionine is known to exacerbate psychopathological symptoms in schizophrenic patients, there is no evidence of similar effects in healthy subjects. The role of methionine as a precursor of homocysteine is the most notable cause for concern. A

“loading dose” of methionine (0.1 g/kg) has been given, and the resultant acute increase in plasma homocysteine has been used as an index of the susceptibility to cardiovascular disease.

## L-Cysteine

Cysteine ( $\alpha$ -amino acid) (Fig.2) is named after cystine, which comes from the Greek word *kustis* meaning bladder. Cystine was first isolated from kidney stones. It is a semi-essential amino acid, which means that it can be biosynthesized in humans. The thiol side chain in cysteine often participates in enzymatic reactions, serving as a nucleophile. The thiol is susceptible to oxidization to give the disulfide derivative cystine, which serves an important structural role in many proteins.



**Fig. 3.** Structure of L-Cysteine

### **Biological Importance**

Due to its ability to undergo redox reactions, cysteine has antioxidant properties. 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. Cysteine is an important precursor in the production of the antioxidant GSH.

### **Toxicity**

Cysteine supplementation is limited by associated toxicity and product instability. Procysteine (l-2-oxothiazolidine-4-carboxylate) is a precursor of cysteine that is inert until metabolized to Cysteine intracellular, thus stimulating glutathione synthesis. The potential toxicities of Cysteine and procysteine were compared in two studies with neonatal rats after a single intravenous administration<sup>18</sup>. In one study, acute high dosage survivorship was compared for approximately equimolar Cysteine dosages of L-Cysteine and Procysteine. Mortality at 7 days after single intravenous dosages of L-Cysteine at 1.52 or 1.14 g/kg or Procysteine at 1.80 or 1.35 g/kg was 80, 50, 10 and 0%, respectively. Clinical pathology parameters and body and organ weights were compared in a second study, following a moderate dosage of Procysteine or equimolar or lower dosages of L-Cysteine. No differences were observed in clinical pathology parameters or body or organ weights at 14 days following single intravenous dosages of L-Cysteine at 369, 185 or 37 mg/kg or Procysteine at 450 mg/kg. Also, procysteine solutions were considerably more stable than L-Cysteine solutions.

## **EXPERIMENTAL**

### ***Preparation of solutions***

All the solutions were prepared in distilled water, through which nitrogen gas was purged for about half-an-hour to expel any dissolved oxygen or carbon dioxide. 'A' grade glassware was used throughout the experimentation and standardized as per the standard procedure.

### ***Materials***

0.05 mol L<sup>-1</sup> Aqueous solutions of L-Cysteine (Cyst), L-Methionine (Met) (E-Merck, Germany) GR grade were prepared by dissolving samples in distilled water. To increase the solubility of the ligands 0.05 mol dm<sup>-3</sup> mineral acid concentration was maintained in the solutions. Carbonate free sodium hydroxide (E-Merck, Germany) pellets were for the preparation of 0.40 mol L<sup>-1</sup> solution. Nitric acid (E-Merck, Germany) of 2.0 mol L<sup>-1</sup> was prepared. Sodium nitrate (E-Merck, Germany) of 2.0 mol L<sup>-1</sup> was prepared to maintain 0.16 mol L<sup>-1</sup> ionic strength in the titrand.

### **2.3. Alkalimetric titrations**

Alkalimetric titrations were carried out using SYSTRONIC (Model 361) digital pH meter with maintaining an ionic strength of 0.16 mol L<sup>-1</sup> with sodium nitrate at ambient condition. Titrations with different ratios of ligand were carried out with 0.4 mol L<sup>-1</sup> sodium hydroxide. Potassium hydrogen phthalate (0.05 mol L<sup>-1</sup>) and borax (0.01 mol L<sup>-1</sup>) solutions were used to calibrate the pH meter. At regular intervals strong acid was

titrated against alkali to check the complete equilibration of the glass electrode.

### **Modeling Strategy**

The computer program SCPHD was used to calculate the correction factor. The best fit chemical model for each system investigated was arrived at using non-linear least-squares computer program, MINQUAD75, which exploits the advantage of constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm. In the present investigation, the data was obtained from linear least squares analysis of the pruned formation data and graphical method. During the refinement of the correction factor and protonation constants of L-Cysteine and L-Methionine were fixed.

### **Refinement model**

MODEL 1 prometaq.txt

TEMP= .0

NK= 3 N= 2 NMBE= 2 NC= 1 NEXP= 3

	FORMATION CONSTANTS		REFINEMENT KEYS	STOICHIOMETRIC COEFFICIENT
( 1)	9.3000	1.9953E 9	1	1 1
( 2)	11.3000	1.9953E 11	1	1 2
( 3)	-13.9000	.1259E-13	0	0 -1

TITRATION NO. 1 EXPT.NO. 1

[MINERAL ACID]= .02317

[LIGAND 1]= .00500

[TOTAL ACID]= .02817

% SOLVENT= .0 TOTAL VOLUME= 50.00

[ALK]= .4031 CF= -.235

TITRATION NO. 2 EXPT.NO. 2

[MINERAL ACID]= .02548  
[LIGAND 1]= .00750  
[TOTAL ACID]= .03298  
% SOLVENT= .0 TOTAL VOLUME= 50.00  
[ALK]= .4031 CF= -.235

TITRATION NO. 3 EXPT.NO. 3

[MINERAL ACID]= .02780  
[LIGAND 1]= .01000  
[TOTAL ACID]= .03780  
% SOLVENT= .0 TOTAL VOLUME= 50.00  
[ALK]= .4031 CF= -.235

69 DATA POINTS HAVE BEEN READ IN AND 400 MAXIMUM WERE EXPECTED

REFINEMENT CONVERGED SUCCESSFULLY

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FUNCTION CALLS= 7  
SUM OF SQUARES= 5.700401716E-06

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BETA(A)	1 1		
		REFINED	8.87578
			.03091
BETA(B)	1 2		
		REFINED	10.99263
			.03619
BETA(C)	0-1		
		CONSTANT	-13.90000

MATRIX OF CORRELATION COEFFICIENTS RHO I,J COLUMN

I	
2	.854
J	1

ARITHMETIC MEAN -3.0665E-07 MEAN DEVIATION 1.7218E-04 STANDARD  
DEVIATION 2.0324E-04

VARIANCE 4.1307E-08 MOMENT COEFFICIENT OF SKEWNESS .08  
MOMENT COEFFICIENT OF KURTOSIS 2.09

OBSERVED CHI-SQUARE IS

7.62 R FACTER=

.017879

SPECIES DISTRIBUTION(%).....

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3.	2.045	45.47	53.65	.00
4.	2.085	48.22	51.89	.00
5.	2.115	49.17	49.38	.00
6.	2.155	51.39	47.07	.00
7.	2.205	54.77	44.71	.00
8.	2.235	54.86	41.79	.00
9.	2.295	58.83	39.03	.00
10.	2.365	63.51	35.86	.00
11.	2.445	68.59	32.22	.00
12.	2.515	71.43	28.56	.00
13.	2.565	71.21	25.37	.00
14.	2.685	77.00	20.81	.00
15.	2.855	84.23	15.39	.00
16.	3.125	92.52	9.08	.00
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25.	2.125	51.43	50.47	.00
26.	2.165	53.89	48.24	.00
27.	2.205	56.15	45.84	.00
28.	2.265	60.62	43.10	.00
29.	2.305	62.35	40.43	.00
30.	2.375	67.21	37.09	.00
31.	2.435	70.41	33.84	.00
32.	2.495	73.07	30.59	.00
33.	2.585	77.97	26.53	.00
34.	2.675	81.78	22.62	.00
35.	2.795	86.59	18.17	.00
36.	2.955	91.87	13.34	.00
37.	3.185	97.15	8.30	.00
38.	3.635	102.42	3.11	.00
39.	7.405	101.46	.00	.00
40.	7.965	92.04	.00	.00
41.	8.265	82.18	.00	.00
42.	8.515	71.18	.00	.00
43.	8.675	61.97	.00	.00
44.	1.985	41.11	55.69	.00
45.	2.015	42.85	54.18	.00
46.	2.045	44.53	52.54	.00

47.	2.075	46.13	50.80	.00
48.	2.105	47.64	48.96	.00
49.	2.145	50.16	47.01	.00
50.	2.185	52.54	44.91	.00
51.	2.225	54.78	42.70	.00
52.	2.275	57.90	40.23	.00
53.	2.315	59.75	37.86	.00
54.	2.375	63.39	34.98	.00
55.	2.415	64.74	32.59	.00
56.	2.485	68.59	29.39	.00
57.	2.565	72.73	25.92	.00
58.	2.705	80.58	20.80	.00
59.	2.845	86.42	16.16	.00
60.	3.035	92.55	11.17	.00
61.	3.265	96.89	6.89	.00
62.	3.515	98.48	3.94	.00
63.	7.275	100.63	.00	.00
64.	7.825	93.65	.00	.00
65.	8.155	85.57	.00	.00
66.	8.315	78.92	.00	.00
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( 4)	-13.9000	.1259E-13	0 -1

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[ALK]= .4031 CF= -.235

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[TOTAL ACID]= .04048  
% SOLVENT= .0 TOTAL VOLUME= 50.00  
[ALK]= .4031 CF= -.235

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[MINERAL ACID]= .02780  
[LIGAND 1]= .01000  
[TOTAL ACID]= .04780  
% SOLVENT= .0 TOTAL VOLUME= 50.00  
[ALK]= .4031 CF= -.235

94 DATA POINTS HAVE BEEN READ IN AND 400 MAXIMUM WERE EXPECTED

REFINEMENT CONVERGED SUCCESSFULLY

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BETA(C)	1 3	REFINED 18.94980	.02922
BETA(D)	0-1	CONSTANT -13.90000	

MATRIX OF CORRELATION COEFFICIENTS RHO I,J COLUMN

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DEVIATION 9.2380E-05

VARIANCE 8.5341E-09 MOMENT COEFFICIENT OF SKEWNESS .05  
MOMENT COEFFICIENT OF KURTOSIS 3.17

OBSERVED CHI-SQUARE IS

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SPECIES DISTRIBUTION(%).....

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3.	1.985	.00	82.99	19.25	.00
4.	2.025	.00	85.52	18.09	.00
5.	2.065	.00	87.48	16.88	.00
6.	2.105	.00	88.89	15.64	.00
7.	2.125	.00	86.70	14.57	.00
8.	2.185	.00	90.12	13.19	.00
9.	2.235	.00	91.27	11.91	.00
10.	2.295	.00	92.88	10.55	.00
11.	2.365	.00	94.61	9.15	.00
12.	2.435	.00	95.29	7.84	.00
13.	2.515	.00	95.75	6.56	.00
14.	2.635	.00	97.53	5.07	.00
15.	2.725	.00	96.10	4.06	.00
16.	2.965	.00	98.69	2.40	.00
17.	3.535	.01	101.34	.66	.00
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20.	7.635	42.31	59.81	.00	.00
21.	7.935	59.99	42.51	.00	.00
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24.	8.845	84.06	7.33	.00	.00
25.	9.285	76.52	2.42	.00	.00
26.	9.455	67.24	1.44	.00	.00
27.	9.735	53.43	.60	.00	.00
28.	1.945	.00	80.43	20.46	.00
29.	1.975	.00	81.68	19.39	.00
30.	2.005	.00	82.67	18.32	.00
31.	2.025	.00	82.04	17.36	.00
32.	2.065	.00	83.88	16.19	.00
33.	2.115	.00	86.53	14.88	.00
34.	2.155	.00	87.51	13.73	.00
35.	2.185	.00	87.09	12.75	.00
36.	2.255	.00	90.27	11.25	.00
37.	2.305	.00	90.90	10.09	.00
38.	2.365	.00	91.82	8.88	.00
39.	2.445	.00	93.45	7.52	.00
40.	2.515	.00	93.66	6.41	.00
41.	2.585	.00	93.25	5.43	.00
42.	2.785	.00	96.96	3.57	.00
43.	2.965	.00	97.46	2.37	.00
44.	3.485	.00	99.73	.73	.00
45.	6.405	3.99	95.91	.00	.00
46.	6.985	13.60	85.85	.00	.00

47.	7.295	24.29	75.13	.00	.00
48.	7.515	34.66	64.60	.00	.00
49.	7.735	47.02	52.81	.00	.00
50.	7.895	55.71	43.28	.00	.00
51.	8.085	65.63	32.92	.00	.00
52.	8.275	73.47	23.79	.00	.00
53.	8.535	81.12	14.44	.00	.00
54.	8.785	83.18	8.33	.00	.00
55.	9.065	80.72	4.24	.00	.00
56.	9.335	73.61	2.08	.00	.00
57.	9.505	65.79	1.25	.00	.00
58.	9.695	56.20	.69	.00	.00
59.	1.955	.00	79.50	19.76	.00
60.	1.975	.00	79.49	18.87	.00
61.	2.005	.00	80.56	17.85	.00
62.	2.035	.00	81.42	16.84	.00
63.	2.085	.00	84.19	15.51	.00
64.	2.105	.00	83.56	14.71	.00
65.	2.165	.00	86.55	13.27	.00
66.	2.205	.00	87.29	12.20	.00
67.	2.235	.00	86.95	11.34	.00
68.	2.285	.00	87.92	10.22	.00
69.	2.345	.00	89.16	9.03	.00
70.	2.435	.00	91.57	7.54	.00
71.	2.535	.00	93.52	6.11	.00
72.	2.605	.00	93.39	5.20	.00
73.	2.675	.00	92.83	4.40	.00
74.	2.885	.00	95.31	2.78	.00
75.	3.185	.00	96.77	1.42	.00
76.	6.565	5.71	94.89	.00	.00
77.	6.995	14.04	86.66	.00	.00
78.	7.145	18.49	80.78	.00	.00
79.	7.385	28.46	71.55	.00	.00
80.	7.555	37.12	63.09	.00	.00
81.	7.635	40.82	57.71	.00	.00
82.	7.705	43.86	52.77	.00	.00
83.	7.955	59.20	40.06	.00	.00
84.	8.085	65.64	32.92	.00	.00
85.	8.285	75.00	23.74	.00	.00
86.	8.385	77.06	19.37	.00	.00
87.	8.635	83.20	11.76	.00	.00
88.	8.895	84.60	6.57	.00	.00
89.	9.075	81.77	4.20	.00	.00
90.	9.285	76.47	2.42	.00	.00
91.	9.395	71.34	1.75	.00	.00
92.	9.555	64.18	1.09	.00	.00
93.	9.625	59.48	.86	.00	.00
94.	9.795	50.78	.50	.00	.00

EXEC.TIME= 0: 0: 0 (H:M:S)

Stop - Program terminated.

## Results and Discussion

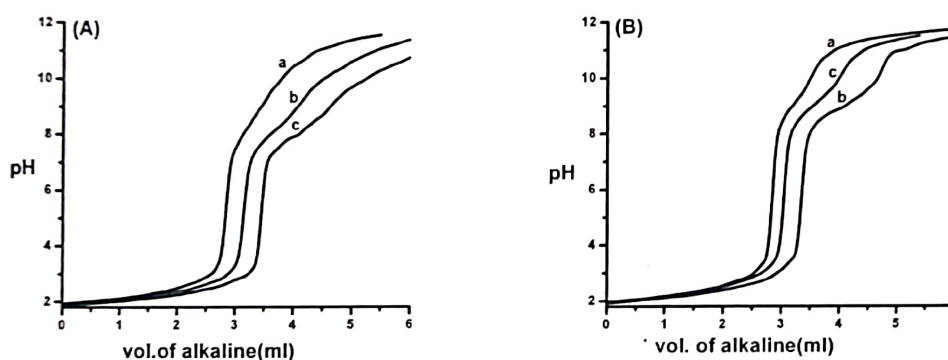
### *Protonation Equilibria of ligands*

Knowledge of the acido-basic properties of amino acids is extremely important in understanding and analyzing the properties of proteins. Further, separation, identification, and quantification of different amino acids and determination of their sequence in proteins are based on their acido-basic behavior. Some amino acids act as tridentate ligands when the third donor site is a nitrogen atom. The additional chelation results in a five or six membered ring.

L- Cysteine (Cyst) and L-Methionine (Met) actively participate in acido-basic equilibria, especially, at physiological pH. Cyst has three functional groups in the order amino followed by one carboxylic and one thiol groups where as Met has only two functional groups which are protonated, amino followed by carboxylic group. Although Cyst has three functional groups, only three protonation constants are reported in the present study because the pH range of study is limited to 2.0-12.0. Hence, Cyst has tridentate and Met has bidentate behavior. Nitrogen donor atoms can associate with hydrogen ions in physiological pH ranges and there is often significant competition between hydrogen and metal ions for the donor sites. This situation results in the simultaneous existence of a number of equilibria, producing an array of successively protonated complexes. So, an understanding of the protonation-

deprotonation equilibria of Cyst and Met is necessary before the metal-ligand equilibria associated with these ligands are investigated.

The alkalimetric titration curves for Cyst and Met in aqueous are shown in Fig. 4. A perusal of the titration curves reveals that the acido-basic equilibria are active in the pH range 2.0-12.0. The computer program SCPHD was used to prune the data obtained in different experiments so that it contains more information than noise and the log  $\beta$ s obtained from the program are used as initial values for final refinement.



**Fig. 4:** Alkalimetric titration curves of (A) Cysteine and (B) Methionine in 50% v/v DOX- water mixtures; a, b, and c indicate 0.250, 0.375 and 0.500 mmol of ligand respectively.

<b>prometaq.txt</b>	0	1.7	0	1.69
1 1.70 9.75	0.1	1.71	0.1	1.7
3 1 2 3	0.2	1.72	0.2	1.71
3 2 2 1 1 3	0.3	1.73	0.3	1.73
1 1 9.30 1	0.4	1.74	0.4	1.75
1 2 11.30 1	0.5	1.76	0.5	1.76
0 -1 -13.9 0	0.6	1.79	0.6	1.78
6.25 0.19768508	0.7	1.81	0.7	1.79
5.0 0.05 0.0 0.0 1	0.8	1.82	0.8	1.81
0.0 0.0 0.0 0.0	0.9	1.85	0.9	1.83
2.5 2.0 36.25 0.0	1	1.86	1	1.85
0.40179895 -.005 0 0	1.1	1.89	1.1	1.87

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prometaq.txt

1 1.70 9.75  
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 3 2 2 1 1 3  
 1 1 9.30 1  
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 0 -1 -13.9 0  
 6.25 0.19768508  
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 2.5 2.31  
 2.6 2.37  
 2.7 2.41  
 2.8 2.78  
 2.9 2.55  
 3 2.62

2.4	2.53	3.1	3.1	3.1	2.73
2.5	2.6	3.2	3.51	3.2	2.82
2.6	2.73	3.3	5.5	3.3	2.9
2.7	2.9	3.4	8.12	3.4	3.15
2.8	3.1	3.5	8.46	3.5	3.41
2.9	3.66	3.6	8.82	3.6	4.38
3	6.85	3.7	9.05	3.7	7.72
3.1	8.5	3.8	9.23	3.8	8.31
3.2	8.94	3.9	9.43	3.9	8.59
3.3	9.11	4	9.66	4	8.77
3.4	9.44	4.1	9.79	4.1	8.96
3.5	9.79	4.2	10.12	4.2	9.08
3.6	10.17	4.3	10.46	4.3	9.22
3.7	10.68	4.4	10.77	4.4	9.39
3.8	10.87	4.5	11.03	4.5	9.5
3.9	11.11	4.6	11.21	4.6	9.67
4	11.26	4.7	11.32	4.7	9.87
4.1	11.37	4.8	11.42	4.8	10
4.2	11.45	4.9	11.48	4.9	10.3
4.3	11.51	5	11.52	5	10.63
4.4	11.56	5.1	11.58	5.1	10.94
4.5	11.6	5.2	11.61	5.2	11.12
4.6	11.65	5.3	11.66	5.3	11.23
4.7	11.68	5.4	11.7	5.4	11.33
4.8	11.72	5.5	11.72	5.5	11.41
4.9	11.75	5.6	11.75	5.6	11.47
5	11.78	5.7	11.77	5.7	11.52
5.1	11.8	5.8	11.8	5.8	11.56
5.2	11.81	5.9	11.82	5.9	11.6
5.3	11.84	6	11.84	6	11.64
5.4	11.86	6.1	11.86	6.1	11.67
5.5	11.88	6.2	11.88	6.2	11.7
5.6	11.89	6.3	11.89	6.3	11.73
5.7	11.9	6.4	11.91	6.4	11.75
5.8	11.92	6.5	11.92	6.5	11.77
5.9	11.93	6.6	11.94	6.6	11.79
6	11.95	6.7	11.95	6.7	11.81
6.1	11.96	6.8	11.96	6.8	11.84
6.2	11.97	6.9	11.97	6.9	11.85
6.3	11.99	7	11.98	7	11.86
6.4	12	7.1	11.99	7.1	11.88
-1,-1		-2,-2		-3,-3	

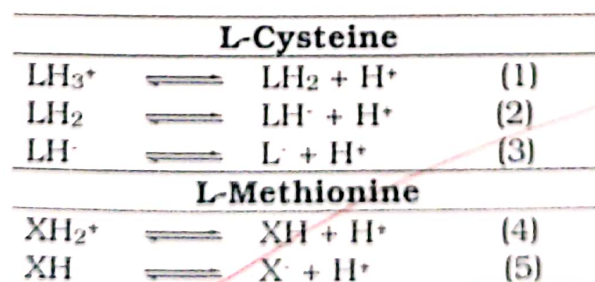
The computer program MINIQAD75 was used to refine protonation constants of the Cyst and Met in aqueous solution. A very low standard deviation in  $\log \beta$  values indicates the precision of these parameters. The small values of  $U$  (the sum of the squares of deviations in concentration of ligand and hydrogen ion at all experimental points) corrected for

degrees of freedom, indicates that the experimental data can be represented by the models.

The presence of influences the protonation-deprotonation of Cyst contains carboxylic, thiol group and amino protons. The different forms of Cyst are  $LH_3^+$ ,  $LH_2$ ,  $LH^-$  and  $L^{2-}$  in the pH ranges 3.0-4.0, 4.0-10.0, 4.0- >11.0 and 9.0-12.0, respectively. Met contains one carboxylic and one amino proton. The different forms of Met are  $XH_2$ ,  $XH$  and  $X^-$  in the pH ranges 2.0-5.0, 4.0-11.0 and 6.0-12.0, respectively.

The protonation-deprotonation equilibria of Cyst and Met are shown in Fig. 4. In these equilibria Cyst and Met can exist in anionic, zwitterionic and cationic forms.

These observations can be explained as follows. When Cyst (Eqs. 1- 3) and Met (Eqs. 4- 5) cations are successively deprotonated, the charge of the species is increased and low dielectric medium favours the protonation reaction, due to dominant electrostatic interactions. Thus, decrease in dielectric constant of the medium should increase the protonation constants.



## Distribution Diagrams

The distribution plots produced using the protonation constants from the best fit models show the formation of  $LH_3^+$ ,  $LH_2$ ,  $LH^-$  and  $L^-$  in the case of Cyst and  $XH_2^+$ ,  $XH$  and  $X^-$  in the case of Met. In both the cases,  $LH_2/XH$  is present to an extent of 90% in the pH range 4.0-10.0. The most protonated species ( $LH_3^+$  in the case of Cyst and  $XH_2^+$  in the case Met) exist in the pH range 2.0-6.0 and 2.0-6.0, respectively.  $LH_3^+$  is deprotonated with increasing pH to form  $LH_2$ ,  $LH^-$  and  $L^-$  in the pH ranges 4.0-10.0 and 6.0-12.0 respectively, in the case of Cyst and  $XH_2^+$  is deprotonated with increasing pH to form  $XH$  and  $X^-$  in the pH ranges 2.0-9.0

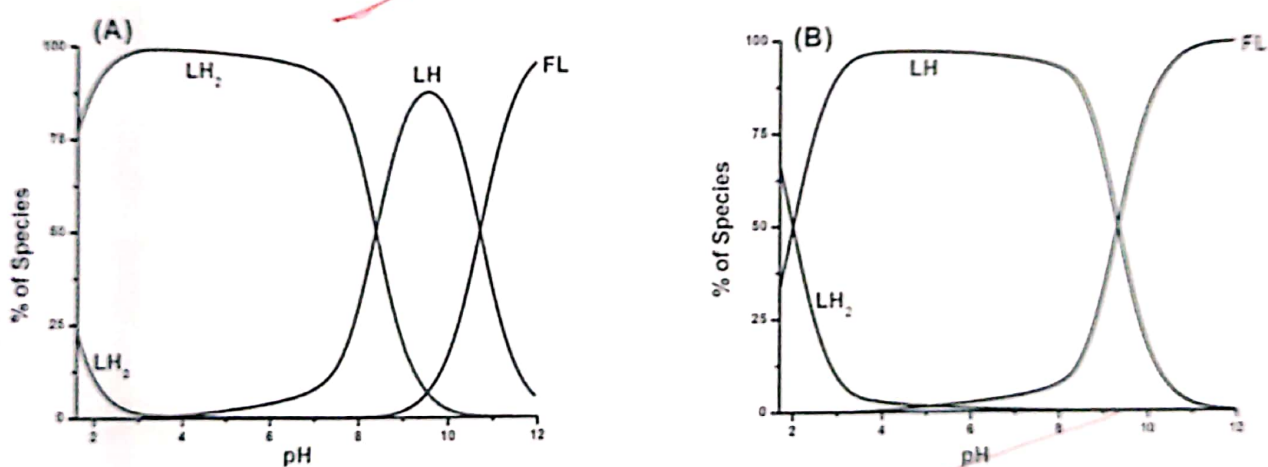
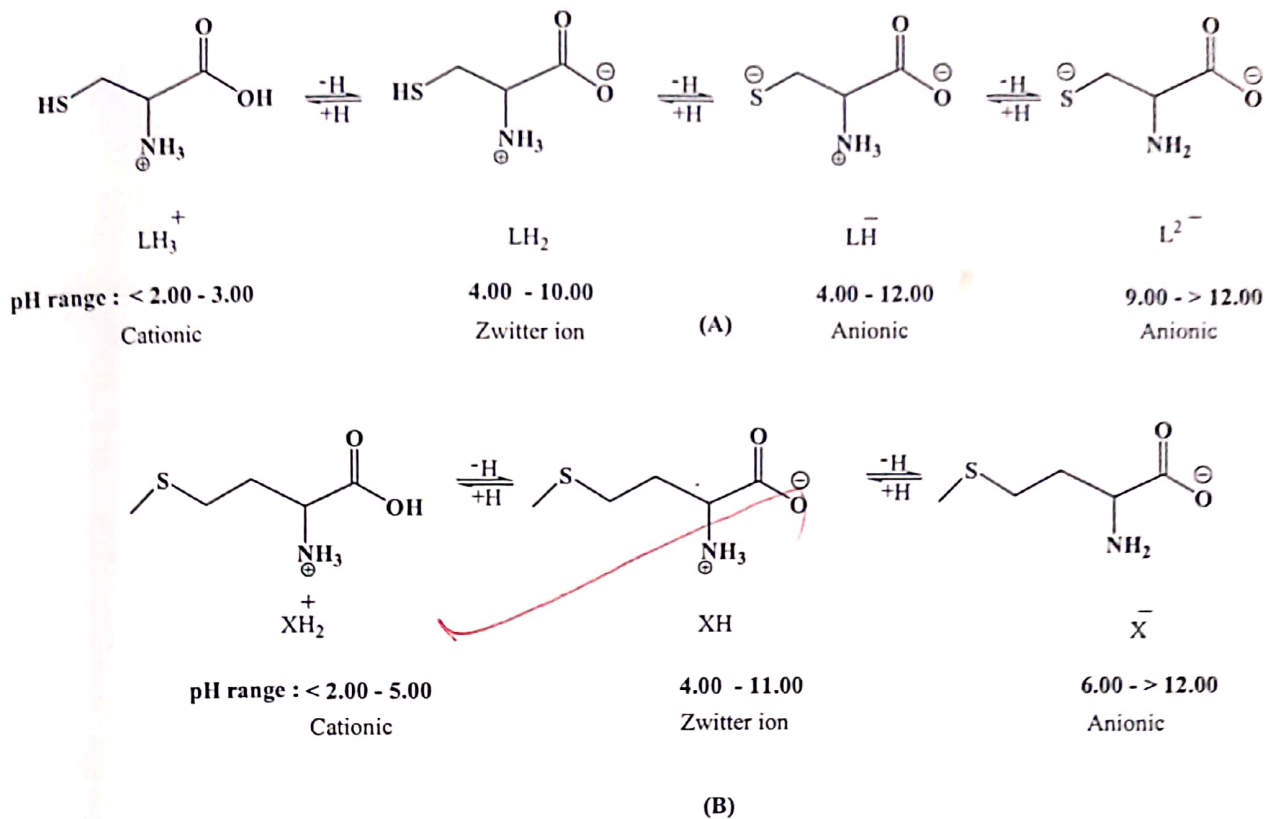


Figure 2: Distribution diagrams of (A) Cyst-(B) Met in aqueous medium



**Figure 4:** Protonation-deprotonation equilibria of (A) Cys and (B) Met

### Protonation Equilibria

1. Different forms of Cyst were  $\text{LH}_3^+$ ,  $\text{LH}_2$ ,  $\text{LH}^-$  and  $\text{L}^{2-}$  in the pH ranges 2.0-3.0, 4.0-10.0, 6.0-12.0 and 9.0-12.0, respectively, and those of Met were  $\text{XH}_2^+$ ,  $\text{XH}$  and  $\text{X}^-$  in the pH ranges 2.0-5.0, 4.0-11.0 and 6.0-12.0, respectively.
2. Cyst formed  $\text{LH}_3^+$  at low pH and deprotonated takes place with the formation of  $\text{LH}_2$ ,  $\text{LH}^-$  and  $\text{L}^{2-}$  with the increase in pH. Met formed  $\text{XH}_2^+$  at low pH and deprotonated to form  $\text{XH}$  and  $\text{X}^-$  successively with the increase in pH.