Electrometric Investigation on Ternary Complexes of Vital Metal Ions with Histamine in TX-100- water medium: A Computer Augmented Study

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Abstract

In human body Chemical speciation shows significant part in comprehension the biological and physiological partsof metal ions and ligands. This study has been revealed that the complexation of Glycylglycine and Histamine with crucial metal ions in Triton X–100 (TX100). The analysis has been carried out using potentiometric method and the outcomesachieved in this examination have been progressed via gran plot method, SCPHD and MINIQUAD75 is utilized for best fit chemical model and refinement of results. In this study effect of surfactant on ternary complexes stability constants, effect of influential parameters like concentration of acid, alkali, ligands and metal ions on the stability constants have been evaluated by conducted an experiment.$MLXH_2$, ML_2XH_2 and MLX_2H_3complexes formed in the present study. Chemical problems were correlated by using the advanced principles of mathematics and fuzzy logic, which is called chemo metrics. Errors in correction factor, ligand concentration and metal concentration less on the stability constants of ternary complexes than other parameters like alkali and acids. Influence of surfactant on stability constants of ternary complexes and effect of surfactant on distribution of metal - ligand species were also explained

Keywords: Glycylglcine, Histamine, Triton X–100 (TX100), Ternary complexes.

Introduction

Chemical Speciation can be defined as an exact form of an element. Chemical Speciation can be used to illustrate its complex structure. Speciation studies are most usually used in medical sciences such as diagnostic, therapeutic and investigative uses of trace elements. Chemical speciation plays vital role in understanding several forms of elemental concentration as this is crucial in neutrino toxicology. Important parameters like pH, ionic strength and temperature will affect the metal-ligand complexes. In the current earlier, researchers made a wide study on the chemical speciation of metal ions with different ligands [1-5]. In living organisms, the ligands in metal containing proteins exist either as non-adjustable form or freely bind to metals in biofluids. These freely bonded ligands be in a reversible stable state with metal ions of a similar kind that happened in biofluids. Previous studies were reported that in biofluids

these simultaneous equilibria associated with different biological ligands as well as metal ions playing significant role [6-9].

In this study, aqueous medium has been employed using Triton X–100 (TX100) and by maintaining ionic strength with sodium chloride solution in order to mimic physiological conditions. Protonation constants of glycylglycine and histamine in SLS– water, CTAB-water and TX-100-water mixtures were reported [10-12]. The present study deals with the stability constants of Ca(II), Zn (II) and Mg(II) in TX100-water mixtures with glycylglycine and histamine.

Experimental

Chemicals and Solutions

0.1 M concentration of Metal ion solutions of Ca (II), Mg (II) and Zinc (II) were prepared using by adding required weight of GR Grade metal salts to deionised water. These solutions were standardized using 0.1 M of EDTA (Merck, India) solution is used to standardized these metal solutions complex metricallyand kept in dried and dark place. 0.05M concentration of the ligand solutions of Glycylglycine (Hi-media India), and Histamine (Spectrochem, India)were prepared by adding appropriate weight of substance deionised water and little amount 0.05 M HCl solution is added in order to increase the solubility.0.2M of HCl (Merck, India) and 0.4M of NaOH (Merck, India) solutions are prepared and standardized with the help of potentiometer using using acid base titrations. To maintain the required ionic strength in titrandwe have used 0.16 M of NaCl (Merck, India) solution. Potassium Hydrogen Phthalate (Merck, India) with 0.05 M concentration and Borax (Merck, India) with 0.01 M concentration solutions were used to calibrate the glass membrane electrode. TX100was used as it is. 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 [13-15].Gran plot method is used to assess the strengths of base and acid. [16-18]

Procedure

A 905 Auto titrandoAnalyzer (Metrohm, Switzerland) (readability 0.001) was used for titrations. The glass electrode was equilibrated by titrating with a strong acid with strong base. After which the electrode has to be equilibrated with surfactant for at least one week before the analysis in order to minimise the errors. The equilibration has to be continued until pH dial readings and calculated pH values become standard. After ensuring the equilibrium of electrode, the titrations were carried out in the medium having different concentrations of TX100-water mixtures (0.0-2.5 % w/v) at 303.0±0.1 K. Titrations were carried out in the presence of different relative concentrations of metal (M) to primary (GG, L) and secondary (Him, X) ligands (M:L:X=1.0:2.5:2.5, 1.0:2.5:5.0 and 1.0:5.0:2.5) with sodium hydroxide. It is important to maintain 1 ml of mineral acid in total volume 50 ml. Finally the titrand present in has to be titrated with 0.4 M NaOH solution. The concentrations of the ingredients are giv1en in Table 1. The details of the experimental procedure and the titration assembly were given elsewhere [19-22].

Table 1: Total initial concentrations of ingredients (in mmol) for mixed - ligand titrations in TX100-watermixtures.

%(v/v)	TM0			TL0		$C_M:C_L:C_X$
TX100	Ca(II)	Mg(II)	Zn(II)	GG	Him	
				0.2488	0.2495	1:2.5:2.5
0.0	0.1023	0.1013	0.1002	0.3767	0.3743	1:5.0:2.5
				0.4990	0.4991	1:2.5:5.0
				0.2498	0.2499	1:2.5:2.5
0.5	0.1023	0.1013	0.1002	0.3747	0.3749	1:5.0:2.5
				0.4996	0.4999	1:2.5:5.0
				0.2493	0.2494	1:2.5:2.5
1.0	0.1023	0.1013	0.1002	0.3739	0.3741	1:5.0:2.5
				0.4986	0.4988	1:2.5:5.0
				0.2484	0.2493	1:2.5:2.5
1.5	0.1023	0.1013	0.1002	0.3726	0.3740	1:5.0:2.5
				0.4968	0.4987	1:2.5:5.0
				0.2493	0.2491	1:2.5:2.5
2.0	0.1023	0.1013	0.1002	0.3739	0.3737	1:5.0:2.5
				0.4986	0.4983	1:2.5:5.0
				0.2498	0.2489	1:2.5:2.5
2.5	0.1023	0.1013	0.1002	0.3747	0.3733	1:5.0:2.5
				0.4996	0.4978	1:2.5:5.0

Modelling strategy

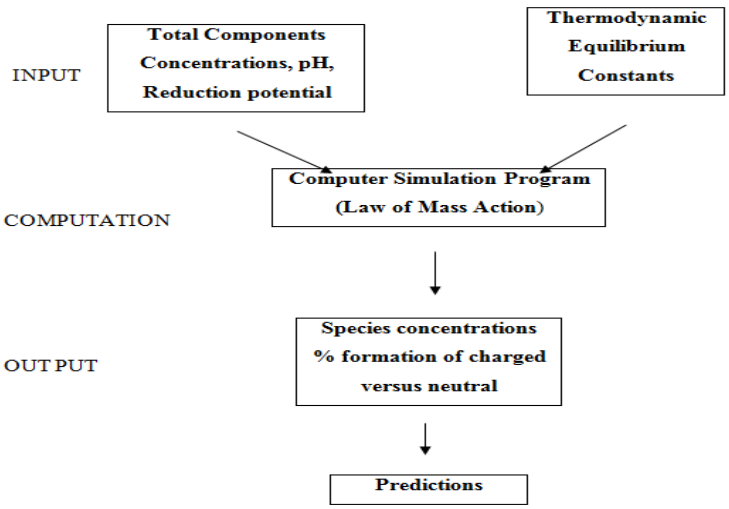

Fig. 1: Basic components of a chemical speciation model

The Modelling strategy of the chemical speciation was shown in Fig.1. Correction factor is one of the main parameter which has to be used to obtain accurate results and can be

calculated the same using a computer program called SCPHD. The stability constants of ternary complexes are calculated by using a computer program MINIQUD75[23-25].

Results and Discussion

After the refinement, the ternary complexes detected were $MLXH_2$, ML_2XH_2 and MLX_2H_3 for Ca (II), Mg(II) and Zn(II) with low standard deviation. The selection the above models done by basing on best fit statistical and chemical consistency with the titration data. The parameters of the best-fit models are given in Table 2. Low standard deviation (SD) in log values of overall stability constants (log β) indicates the precision of the parameters.

Residual analysis

The differences between the experimental data and the data simulated based on the model parameters are called residuals. The statistical parameters like $\chi 2$, Skewness, Kurtosis, U_{corr} and R-factor were in the acceptable range and which indicates the precision of the model. The $\chi 2$ values obtained the present study are ranging from 7.82-56.85 for Ca(II), 8.00-37.45 for Mg (II) and 5.54-43.67 for Zn(II). The values obtained in the present study reveals that the model is accepted as the values are less than the table value. The R values obtained in the present study are ranging from 0.0104-0.0384, if the R values are greater than the Rtable values, the model will be rejected. In the present study the obtained values are less than the table values, which indicates the acceptance of model.The small values of U indicates that experimental data can be represented by the model. The model is said to be adequate when the statistical parameters of the residuals and errors are not significantly different. The residuals like χ^2, skewness, kurtosis and R-factor are tested normal distribution with respective to hypothesis of least squares analysis. For an ideal normal distribution, kurtosis value should be three, which is called mesokurtic. If the value is less than three, the kurtosis value shows that the residuals are in leptokurtic pattern.It is platykurtic, when the value is less than three and leptokurtic, when it is more than three. In the present study the model is platykurtic for Ca(II) and Mg(II) and mesokurtic for Zn(II) models. Skewness values in the present study are from -0.65 to 0.84 for Ca(II), 0.07 to 1.58 for Mg(II) and 0.36 to 1.34 for Zn(II).From this data we conclude that the residuals are in normal distribution and the least squares method has been applied for this data. The obtained statistical parameters in the present investigation are described below.

Table 2: Best fit chemical models of GG-M(II)-Him complexes in TX100-watermixtures.

TX100 % v/v	log β_{mlh}(SD) $MLXH_2$	ML_2XH_2	MLX_2H_3	pH-Range	NP	U_{corr} *10^8	χ^2	Skew-ness	Kurt-osis	R-factor
Ca(II)										
0.0	25.09(27)	27.67(47)	35.34(12)	2.4-8.0	22	3.28	7.82	0.84	6.65	0.0104
0.5	24.70(26)	26.78(35)	35.37(7)	2.4-8.0	20	1.75	9.80	0.06	4.39	0.0142
1.0	25.10(22)	27.31(29)	35.62(45)	2.5-9.5	33	18.97	56.85	-0.34	1.92	0.0308
1.5	24.56(20)	26.89(33)	34.95(17)	2.5-8.6	23	6.68	12.23	-0.45	3.10	0.0186
2.0	24.39(27)	26.66(39)	35.23(7)	2.5-8.6	20	0.77	2.80	-0.65	3.18	0.0286
2.5	24.30(20)	26.61(25)	35.38(23)	2.6-9.0	27	4.18	52.47	1.11	8.05	0.0134
Mg(II)										
0.0	26.43(31)	29.19(34)	36.92(24)	2.4-8.0	33	15.18	37.45	0.30	3.95	0.0250
0.5	25.61(23)	29.31(16)	35.75(20)	2.7-8.0	26	0.19	8.00	1.58	11.44	0.0135
1.0	25.53(33)	28.97(28)	35.05(29)	2.5-8.5	47	17.93	34.47	0.07	3.62	0.0208
1.5	25.58(17)	28.60(24)	34.43(35)	2.8-8.3	36	11.94	17.78	0.68	5.05	0.0181
2.0	25.11(35)	28.72(38)	33.92(44)	3.2-8.3	31	24.09	26.58	0.65	4.23	0.0282
2.5	24.64(31)	27.20(40)	33.75(33)	2.7-8.2	22	28.45	26.00	0.73	2.3.5	0.0296
Zn(II)										
0.0	24.40(22)	27.83(36)	34.38(35)	3.0-8.4	36	8.29	43.67	1.34	4.79	0.0304
0.5	25.53(29)	28.70(44)	35.22(32)	3.0-7.6	37	27.24	21.95	0.66	3.63	0.0321
1.0	26.24(25)	29.89(37)	35.11(38)	2.8-8.0	53	9.37	35.32	0.36	3.17	0.0314
1.5	26.22(16)	29.97(34)	35.35(52)	3.0-8.2	63	10.09	40.38	0.40	3.15	0.0278
2.0	26.39(26)	30.30(32)	35.53(26)	2.9-8.0	48	17.75	14.00	0.45	3.58	0.0259
2.5	26.54(30)	30.90(42)	35.65(35)	2.5-7.3	25	39.86	5.54	0.64	2.97	0.0384

U_{corr} = U/ (NP-m) X108, where m = number of species; NP = Number of experimental points; SD = Standard deviation.

Effect of Surfactant on the Stability of Ternary Complexes

The linear variation in the stability constants of with respective to mole fraction of TX100-water mixtures is due to the polarity. The deprotonated carboxyl ions are stabilized compared to the protonated forms by the cationic micelles due to the formation of ion pairs. The linear variations of stability constants (log β) with the mole fraction ofTX100 (Fig.2) indicate the dominance of electrostatic forces over non-electrostatic forces[26-29].

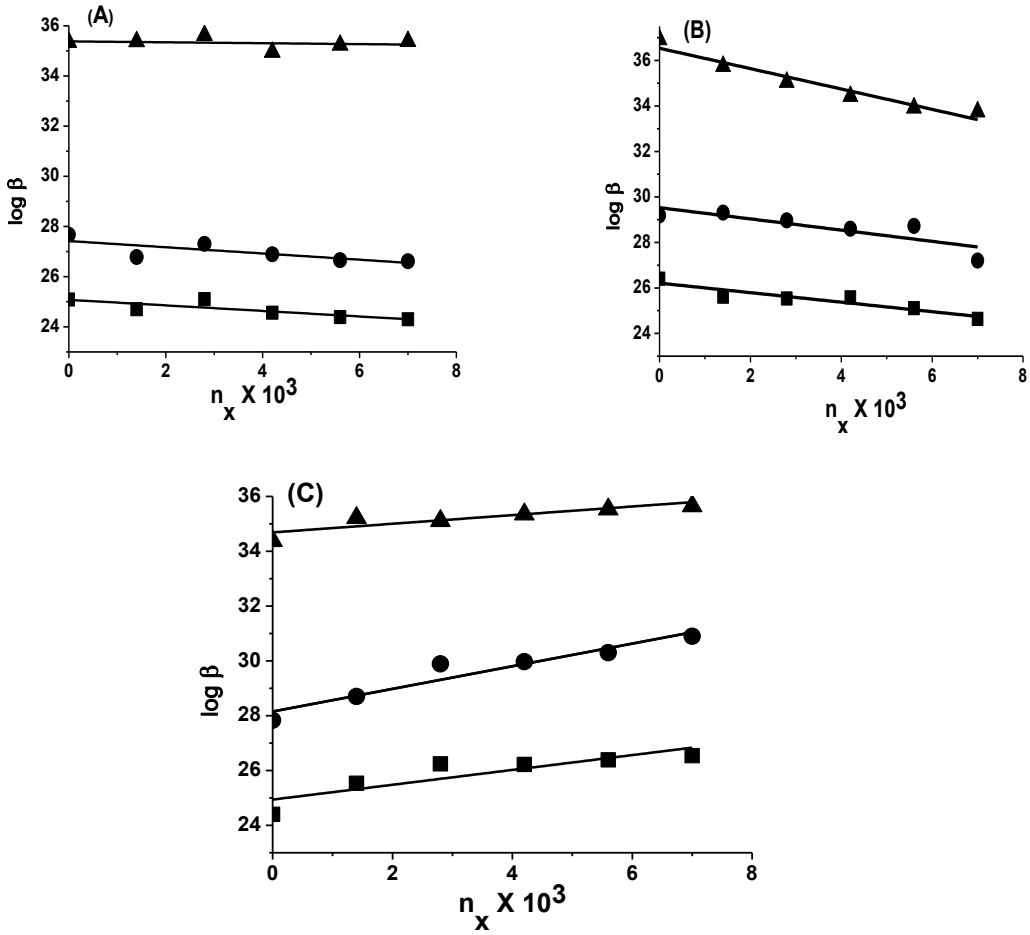

Fig. 2: Variation of stability constants of ternary complexes with mole fraction of TX100. (A) Ca (II), B) Mg (II) and C) ZnII) (■) logβ_{MLXH2}, (●)logβ_{ML2XH2} (▲)logβ_{MLX2H3}.

Stability of Ternary Complexes

The log X and ΔlogK values calculated (Table 3) from binary and ternary complexes are included in Table 4. In the present study, the log X values range from 8.23 to 14.52 and some values found to be higher than those expected on statistical base (0.6). The higher values account for the extra stability of the ternary complexes. The values of the Δ logK ranging from -5.15to 5.02for Ca(II), Mg(II) and Zn(II). The change in the stability of the ternary complexes as compared to their binary analogues was quantified based on the difference in stability (Δlog K) for the reactions ML with X and $M_{(aq)}$ with L and X [30-35]

Table 3: Calculation of log X and ΔlogK values from overall stability constants.

$$\Delta \log K_{ML2XH2} = \log \beta_{ML2XH2} - \log \beta_{ML2H} - \log \beta_{MXH}$$
$$\Delta \log K_{MLX2H3} = \log \beta_{MLX2H3} - \log \beta_{ML2H2} - \log \beta_{MX2H}$$
$$\log X_{MLXH2} = 2\log \beta_{MLXH2} - \log \beta_{ML2H2} - \log \beta_{MX2H}$$

Table 4: ΔlogK and log Xvaluesof GG-M(II)-Him ternary complexes in TX100-water mixtures.

% v/v TX100	Δlog K_{ML2XH2}	Δlog K_{MLX2H3}	log X_{MLXH2}
	Ca(II)		
0.0	2.71	-2.57	12.27
0.5	2.38	-2.58	11.45
1.0	2.34	-4.24	10.34
1.5	2.33	-4.52	9.65
2.0	1.67	-4.81	8.74
2.5	1.23	-4.99	8.23
	Mg(II)		
0.0	2.82	-4.06	11.88
0.5	3.15	-4.11	11.36
1.0	3.26	-4.60	11.41
1.5	2.51	-5.15	11.58
2.0	3.47	-4.25	12.05
2.5	2.53	-4.38	11.15
	Zn(II)		
0.0	2.53	-3.68	10.74
0.5	3.3	-2.53	13.31
1.0	4.34	-3.70	13.67
1.5	4.25	-3.36	13.73
2.0	4.66	-3.28	13.97
2.5	5.02	-2.91	14.52

Effect of Influential Parameters on Stability Constants

The change in the concentration of alkali, acid, correction factor, metal and ligands plays important role in errors in the study. Change in their concentration will lead to change in the values of stability constants and the standard deviation. This effect of the parameters on stability constants were given in Table 5. The present study predicts that the major errors are due to errors in alkali and acid. This indicates the appropriateness of the experimental conditions and accuracy of the concentrations

Table 5: Effect of errors in influential parameters on the stability constants of ternary complexes of Zn(II) with GG and Him in 2.0% v/v TX100 water mixture.

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Ingredient	% Error	log β(SD)		
		$MLXH_2$	ML_2XH_2	MLX_2H_3
Alkali	0	25.52(25)	28.65(33)	35.60(30)
	-5	Rejected	Rejected	36.82(36)
	-2	26.13(35)	Rejected	38.17(68)
	2	26.27(45)	Rejected	35.64(32)
	5	24.81(62)	28.26(38)	Rejected
Acid	-5	Rejected	Rejected	38.10(88)
	-2	Rejected	28.81(38)	36.23(48)
	2	26.31(52)	29.36 (53)	36.34(51)
	5	Rejected	Rejected	36.98(67)
GG	-5	25.44(28)	28.60(36)	35.93(35)
	-2	25.57(26)	28.22(35)	35.72(32)
	2	25.47(27)	28.88(37)	35.87(33)
	5	25.51(25)	28.45(34)	35.63(31)
Him	-5	25.21(24)	28.74(37)	35.21(36)
	-2	25.18(24)	28.30(34)	35.23(36)
	2	25.29(26)	28.36(34)	35.27(35)
	5	25.58(28)	28.52(35)	35.26(35)
Metal	-5	25.77(27)	28.70(34)	35.39(32)
	-2	25.45(26)	28.50(35)	35.98(34)
	2	26.01(28)	28.89(38)	35.70(33)
	5	26.02(28)	28.93(39)	35.81(33)

Chemical Speciation

The distribution diagrams drawn using the formation constants of the best fit model are shown in Fig.2 which contain protonated and unprotonated species like$MLXH_2$, ML_2XH_2 and MLX_2H_3 for Ca(II), Mg(II) and Zn(II). The formation of the possible complex species can be represented by the following equilibria.

$M(II) + LH_2^{+} + XH_2^{2+} \rightleftharpoons MLXH_2^{3+} + 2H^{+}$ ------ (1)

$MLXH_2^{3+} \rightleftharpoons MLXH^{2+} + H^{+}$ ------ (2)

$MLXH^{2+} \rightleftharpoons MLXH^{+} + H^{+}$ ------ (3)

$MLXH \rightleftharpoons MLX^{-} + H^{+}$ ------ (4)

$M(II) + 2LH_2^{+} + XH_2^{2+} \rightleftharpoons ML_2XH_3^{3+} + 3H^{+}$ ------ (5)

$ML_2XH_3^{3+} \rightleftharpoons ML_2XH_2^{2+} + H^{+}$ ------ (6)

$ML_2XH_2^{2+} \rightleftharpoons ML_2XH^{+} + H^{+}$ ------ (7)

$ML_2XH^{+} \rightleftharpoons ML_2X^{+} + H^{+}$ ------ (8)

$M(II) + LH_2^{+} + 2XH_2^{2+} \rightleftharpoons MLX_2H_3^{4+} + 3H^{+}$ ------ (9)

$MLX_2H_3^{4+} \rightleftharpoons MLX_2H_2^{3+} + H^{+}$ ------ (10)

$MLX_2H_2^{3+} \rightleftharpoons MLX_2H^{2+} + H^{+}$ ------ (11)

$MLX_2H^{2+} \rightleftharpoons MLX_2^{+} + H^{+}$ ------ (12)

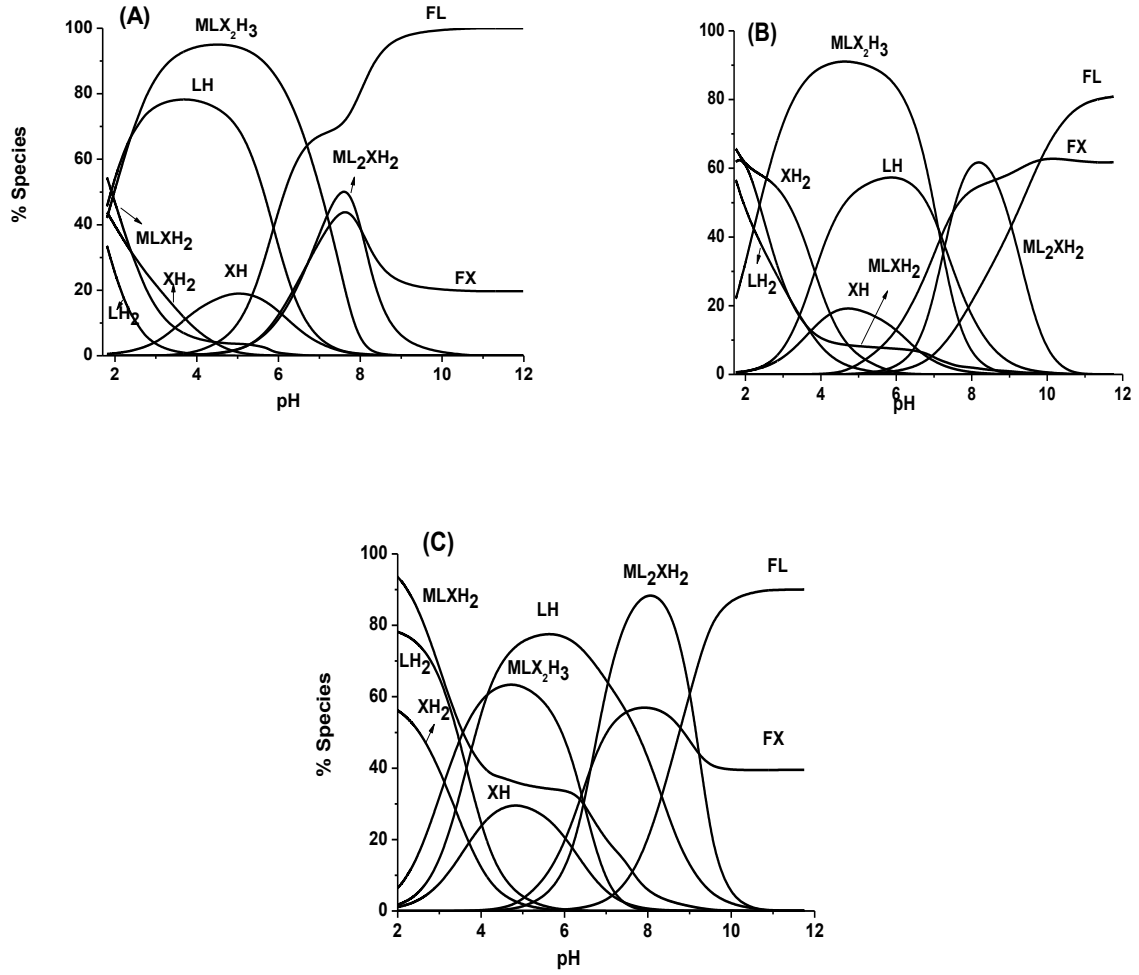


Fig. 3: Distribution diagrams for ternary complexes of GG and Him with (A) Ca(II), (B) Mg(II) and (C) Zn(II) in 1.0% v/v TX100-water mixtures.

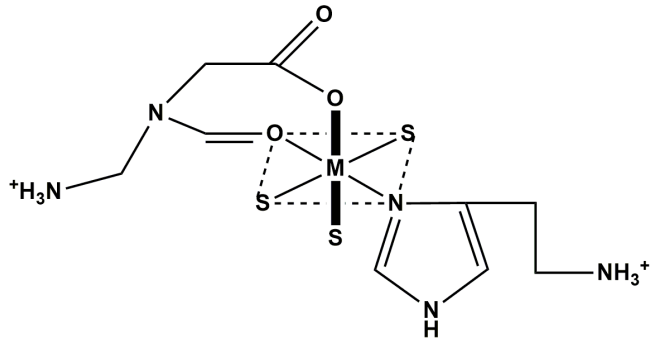
In the pH range 2.0-10.5, 5-hydroxysalicylic acid forms complexes with metal ions of calcium, magnesium and zinc, which divalent. The metal ligand complexes formed in the present study are ML_2H_3, ML_2H_4, and ML_3H_4. It is observed that there is a measurable change in the stability constants of the metal complexes with respective to CTAB composition, it describes the predominant role of electrostatic forces. As the concentration of CTAB is increasing, the log_{mlh} values of Ca(II), Mg(II) and Zn(II) complexes were increased. Stabilization and destabilization of species are caused by electrostatic interactions and a decreasing dielectric constant. The stability of reactant modified complexes is in the order alkali component > acid component > ligand component > metal component > total volume component > log F component. This study contributes to our understanding of the interactions between metals and ligands in aqueous-organic solvent combinations and has the potential to be extremely instructive for future research on medicinal applications.

In the pH range, 1.8-10.0, Glycylglycine and Histamine forms complexs with metal ions of calcium, magnesium and zinc, which divalent.The metal ligand complexes formed in the present study are$MLXH_2$, MLX_2H_3 and ML_2XH_2.

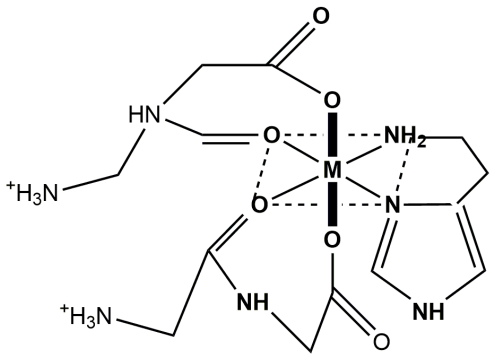
The ternary species formed in the present study are $MLXH_2$, MLX_2H_3 and ML_2XH_2 in the 1.8-10.0 pH range. The refined species are same for Ca(II), Mg(II) and Zn(II). In the first metal ion reacts with LH_2^+ and XH_2^{2+}at low pH to form MLXH2which can be seen in Equilibrium 1. When the pH is increasing, the percentage of MLXH2decreases and it is continued up to pH 5.0. The formation of MLX_2H_3 can be observed with highest percentage of species at pH 6.0, which is formed by the metal ligand interaction i.e. $LH_2^+$and $2XH_2^{2+}$, this can be understood with the equilibrium 9. There by adding base to the solution, pH increases, the increase in the pH reduces the percentages MLX_2H_3species. At pH 5.0, the metal ligand interaction leads the formation of ML_2XH_2 and shows highest percentage of species at pH 8.0, which can be understood through Equilibrium 6.A perusal of the distribution diagrams (Fig. 3) reveals that the concentrations of mixed ligand complexes are less at very low pH and increases with high pH.

Proposed Structures

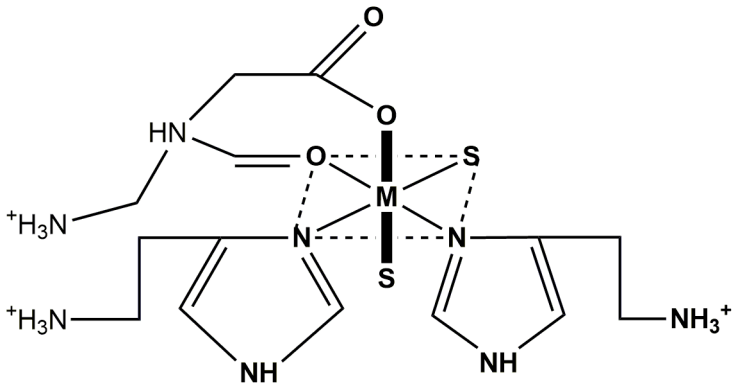
The literature suggests that Ca(II), Mg(II) and Zn(II) complexes shall be octahedral [25, 26].The proposed structures are based on the above equilibria presented in Fig. 4 and literature review.



$MLXH_2$



ML_2XH_2



MLX_2H_3

Fig. 4: Structures of ternary complexes of GG and Him with Ca(II), Mg(II) and Zn(II), where Sis either solvent or water molecule.

Conclusions

The ternary metal complex species detected are $MLXH_2$, ML_2XH_2 and MLX_2H_3 for Ca(II), Mg(II) and Zn(II). Where L = GG and X = Him. Only these species are refined due to the restricted pH ranges and the possible active forms of ligands like LH_2^+, LH, and XH_2^{2+}, XH^+

for GG and Him, respectively.The extra stability of ternary complexes over binary complexes can be understood with the help of ΔlogK values.The values of statistical parameters reveal the adequacy of the model.The linear variation in the stabilities of ternary complexes with increasing mole fraction of the surfactant is due to the dominance of electrostatic forces over equilibrium process.Errors in alkali an acid impacts more on the stability constants of ternary complexes than other parameters like correction factor, lignd concentration and metal concentration.

Conflict of Interest Statement:**The Authors Have No Conflict of Interest**

References:

1. Balakrishna,M., Srinivasa Rao,G., Ramanaiah,M., Nageswara Rao, G. and Rama Raju,B., 2017. pH metric investigation on Chemical Speciation of Co(II), Ni(II) And Cu(II) Complexes With 5-Hydroxysalicylic Acid In Urea-Water Mixtures, *J. Indian Chem. Soc.*,94, p.905-912.
2. Ramanaiah, M., Gowri Kumari, V. and Sailaja, B.B.V., 2016. Effect of Anionic Surfactant on the Protonation Equilibria of L-Phenylalanine and Maleic Acid. *J. Indian Chem. Soc.*, 93, p.285-292.
3. Balakrishna, M. and Seetharm, P., 2020. Qualitative and Quantitative Analysis of Metals in Vital Withania Somnifera Medicinal Plant Using Inductively Coupled Plasma-Mass Spectroscopy, *Plant Cell Biotechnology and Molecular Biology*, 5, p.136-43.
4. Ramanaiah, M., Goutham Sri, S. and Sailaja, B.B.V., 2014. Stability of Binary Complexes of Pb(II), Cd(II) and Hg(II) with Maleic acid in TX100-Water Mixtures. *Bull. Chem. Soc. Ethiopia.* 28(3), p.383-391.
5. Bunton, C.A., Cerichelli, G., Ihara, Y. and Supulveda, L., 1979. *J. Am. Chem. Soc*., 101, p.2429.
6. Feakins, D., Neille, R.D.O. and Woghonie, W.E, J., 1983. *Chem. Soc. Faraday Trans*., 35, p.2289.
7. Gran, G., 1952. Determination of the equivalence point in potentiometric titrations. Part II, *The Analyst*, 77, p.661-671.
8. Gran, G., 1988. Equivalence volumes in potentiometric titrations, *Anal Chim Acta*, 206, p.111-123.
9. Gans P., Sabatini A. and Vacca A., 1976. *Inorg. Chim.Acta*., 18, p.237.
10. Nageswara Rao, Ch., Ramanaiah, M. and Sailaja, B.B.V., 2014. Influence of Dielectric Constant on Protonation Equilibria of Maleic Acid and L-Asparagine in Acetonitrile Water -Mixtures. *Chem. Speciat. Bioavail.*, 26(4), p.266-272.
11. Ramanaiah, M.,Seetharam,P., Balakrishna,M. and Rama Raju,B., 2019. Potentiometric Studies of ComplexeEqulibria of Ca^{II}, Mg^{II} and Zn^{II} with 5-Sulphosalicylic Acid in Cationic Micelles of CTAB, *Heliyon*, 2019, 5(8), p.e02157.
12. Balakrishna, M., Srinivasa, Rao G., Ramanaiah, M., Nageswara Rao, G. and Rama Raju B., 2017. Speciation studies of ternary complexes of Co(II), Ni(II), and Cu(II) with 5-Sulfosalicylic acid and 5-Hydroxysalicylic acid in urea-water mixtures. *Resea. J. Phar. Tech.*, 10(11), p.3681-3686.
13. Ramanaiah,M., Gouthamsri,S., Balakrishna,M. and Rama Raju,B., 2017. Effect of Cationic Micelles of Cetyltrimethylammonium Bromide on Protonation Equilibria of Salicylic Acid Derivatives. *J. Chilean Chem. Soc.*, 62(4), p.3677-3682.

14. Balakrishna, M., Ramanaiah, M., Ramakrishna, B., Jayanthi Rao, M. and Neeraja, R.2022. Inductively Coupled Plasma-Mass Spectroscopy: Machine Learning Screening Technique for Trace Elemental Concentrations in Hemidesmus Indicus, Annals *of Forest Research*, 65(1) p.4431-4445.
15. Ramanaiah,M., Gouthamsri,S., Balakrishna,M.,Rama Raju,B. and Sailaja,B.B.V., 2017. Effect of Nonionic Micelles of TritonX-100 on Protonation Equilibria of Salicylic Acid Derivatives. *J. Indian Chem. Soc.,* 94, p.253-259.
16. Balakrishna, M. and Seetharm, P., 2021. Qualitative and Quantitative Evaluation of Trace Elements in Amaranthaceae Family Medicinal Plant Using ICP-MS, International Journal of *Current Research and Reviews*, 13(5), p.58-65.
17. Ramanaiah, M., Seetharam, P. and Sailaja, B.B.V., 2014. Chemical speciation of ternary complexes of Pb-II, Cd-II and Hg-II with L-phenylalanine and maleic acid in TX100-water mixtures,*J. Ind. Chem. Soc.*, 91(6), p.1011-1020.
18. Gowri Kumari, V., Ramanaiah, M. and Sailaja, B.B.V., 2015. A Speciation of Binary Complexes of Co(II), Ni(II) and Cu(II) With L-Phenylalanine In Anionic Micellar Medium. *Chem. Speciat. Bioavail.,* 27(3), p.121-126.
19. Ramanaiah, M., Nageswara Rao, Ch. and Sailaja, B.B.V., 2014. Study of Ternary Complex Stability Constants of PbII, CdII, and HgII with L-Phenylalanine and Maleic Acid in SDS–Water Mixtures. *Proc. Nat. Acad. Sci., India,*84(4), p.485-494.
20. Balakrishna, M. and Srinivasa Rao, G., 2017. Study of Ternary Complex Stability Constants of Co(II), Ni(II) and Cu(II) with 5-Sulfosalicylic acid and 5-Hydroxysalicylic acid in DMF-Water Mixtures, *Res. J. Chem. Env.*, 21, p.20.
21. Ramanaiah, M. and Sailaja, B.B.V., 2014. Mixed Ligand Complex Formation Equilibria of Some Toxic metal Ions with L-Phenylalanine and Maleic Acid in CTAB-Water *Mixtures. J. Indian Chem. Soc.,* 91, p.1649-1660.
22. Balakrishna, M., Ramanaiah, M., Nageswara Rao, G., Ramaraju, B. and Srinivasa Rao, G., 2017. Influence of Dielectric Constants on Protonation Equlibria of 5-Sulfo Salicylic Acid and 5- Hydroxy Salicylic Acid in Urea-Water Mixtures. *J. Indian Chem. Soc.,* 94, p.37-45.
23. Ramanaiah, M. and Sailaja, B.B.V., 2014. Protonation Equilibria of L-Phenylalanine and Maleic Acid in Cationic Micellar Media. *Chem. Speciat. Bioavail.,* 26(2), p.119-125.
24. Seetharam, P., Ramanaiah, M., and Sailaja, B.B.V., 2016. Protonation Equilibria of glycylglycine and Histamine in cationic micellar media, *J. Ind. Chem. Soc*., 93(8), p.929-936.
25. Ramanaiah, M., Goutham Sri, S. and Sailaja, B.B.V., 2014. Formation of Binary Complexes of Pb(II), Cd(II) and Hg(II) With Maleic acid in CTAB-Water Mixtures. *Chem. Speciat. Bioavail.*,26(4), p.231-239.
26. Seetharam, P., Ramanaiah, M., Sathish Mohan, B. and Sailaja, B. B. V., 2020. Surfactant Effect on Acido-Basic Equilibria of Glycylglycine and Histamine in Neutral Micellar Media, *Res. J. Chem. Env.*, 24, p.57-62.
27. Ramanaiah, M. andSailaja, B.B.V., 2014. pH-metric Investigation on Binary Complexes of Pb(II), Cd(II) and Hg(II) with maleic acid In SLS-Water Mixtures. *J. Indian Chem. soc.*, 91, p.639-645.

28. Nageswara Rao, Ch., Ramanaiah, M. and Sailaja, B.B.V., 2016. Speciation of Binary Complexes of Pb(II) and Cd(II) With L-Asparagine in Dimethyl sulfoxide Medium. *Bull. Chem. Soc. Ethiop*. 30(1), p.71-78.
29. Ramanaiah, M. and Sailaja, B.B.V., 2014. pH-metric Investigation on Binary Complexes of Pb(II), Cd(II) and Hg(II) with maleic acid In SLS-Water Mixtures. *J. Indian Chem. soc.,* 91, p.639-645.
30. Seetharam, P., Balakrishna, M., Sathish Mohan, B., Satyanarayana, G. and Sailaja, B. B. V., 2021. Potentiometric studies on Bioactive Material Species of Ternary Complexes, *Russian Journal of General Chemistry*, 90(12), p.2467-2472.
31. Ramanaiah, M., Goutham Sri, S. and Sailaja, B.B.V., 2014. Chemical Speciation of Pb(II), Cd(II) and Hg(II) Binary Complexes of L-Phenylalanine in CTAB -Water Mixtures. *J. Indian Chem. soc.,* 91, p.351-357.
32. Seetharam, P., Ramanaiah, M. and Sailaja, B.B.V., 2016. pH- metric Investigation on Binary Complexes of Ca(II), Mg(II) and Zn(II) with Glycylglycine in TX100 – Water Mixtures, *Der Pharma Chemica*, 8(13), p.91-98.
33. Seetharam, P., Balakrishna, M., Ramanaiah, M. and Sailaja, B.B.V., 2021. Potentiometric Studies on Bioactive Material Species of Ternary Complexes in SLS-Water mixtures, *Materials Today: Proceedings*, 42, p.3046-3053.
34. Ramanaiah, M., Balakrishna, M., Ramakrishna, B., Venkataramana, M. and Prasada Rao, B.V.V., 2022. Profiling of Trace Elements in Indian Vital Medicinal Plant Momirdica Charantia Using Inductively Coupled Plasma-Mass Spectroscopy. Annals of Forest Research 65(1):3039-3048.
35. Seetharam, P., Ramanaiah, M., RamuNaiudu, P. and Sailaja, B. B. V., 2017. Speciation of Binary Complexes of Ca(II), Mg(II) and Zn (II) with Histamine in CTAB Water Mixtures, *Res. J. Pharm. Biol. Chem. Sci.,* 8 (2), p.1116-1125.