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Speciation of binary Complexes of Ca (II), Mg (II) and Zn (II) with Histamine in CTAB-Water Mixtures.

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ABSTRACT

The formation constants of binary complexes of Ca(II), Mg(II) and Zn(II) with Histamine in (0.0-2.5% *v/v*) CTAB-water mixture were determined pH metrically at 303.0 K and an ionic strength of 0.16 mol L^{-1}. The existence of various binary complexes was established from modeling studies using the computer program MINIQUAD75. The best fit chemical models were arrived at based on the statistical parameters like crystallographic R factor and sum of squares of residuals in mass-balance equations. The trend in the variation of stabilities of binary complexes with change in the mole fraction of the medium was explained based on electrostatic and non-electrostatic forces. The distribution diagrams of the complex species are also presented.
Keywords: Formation constants, Binary Complexes, Histamine, CTAB, pH.

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INTRODUCTION

Speciation analysis is important in human biology, nutrition, toxicology and in clinical practice. The speciation study of essential metal ion complexes is useful to understand the role played by the active site cavities in biological molecules and the bonding behaviour of protein residues with the metal ion. The species refined and their relative concentrations under the experimental conditions represent the possible forms of amino acids in bio-fluids.

Histamine is a biologically active compound as a biogenic ammine which is naturally formed in essentially all mammalian tissues. The physiological and pathological processes in which histamine is involved may be summarized as follows: It mediates a wide range of cellular responses, including allergic and inflammatory reactions, gastric and secretion and neurotransmission in parts of the brain. It is also related to blood pressure, vasodilatation, heart stimulation, intestine and bronchi, regulation of sleep as well as to the concentration of the smooth muscle of gut [1-4]. The contribution of histamine in these physiological and pathological processes and its use in pharmacology make it a focal point for the researchers in biochemistry. The structure of histamine consists of an imidazole heterocycle with an ethylamine side-chain and exists in two tautomeric forms (N^{π}-H and N^{τ} - H) in aqueous solution[5]. Histamine forms complexes with biologically metals and most of its active compounds are those forming are six-membered ring through the side chain of nitrogen, nitrogen of an aromatic nucleus and a polar center[6]. In this context, the transition metal ions, especially Cu (II) and Ni (II) play an important role in histamine and antihistamine activity by changing the pharmacological effects of this molecule [5]. Metal Histamines Besides the earth alkaline metals Mg (II), Ca (II) and Cd (II) have been studied with respect to histamine complexation [7, 8]. The most frequently reported speciation schemes are a series of ML, -complexes with n=1, 2, 3. In these complexes L acts as a bidentate ligand forming a six membered ring with the metal ion. Furthermore, protonated species, MHL and MHL_2, have been proposed in the alkaline earth's system [7] and with Ni (II) [9], Cu (II) [10-12], Zn (II) [9] and Cd (II) [8]. In these complexes data clearly indicate the presence of protonated imidazole nitrogen, hence the metal ion is monodentately coordinated to the amino group.

Calcium is an important component of a healthy diet and a mineral necessary for life. Calcium is present in the animal's skeleton, teeth, egg shell and in the corals [13]. Calcium plays an important role in human body, in building stronger, denser bones early in life and keeping bones strong and healthy later in life. Calcium deficiency leads to rickets and poor blood clotting. An overlooked source of calcium is egg shell [14-15]. Magnesium plays a role in the stability of all polyphosphate compounds in the cells, including those associated with ATP, DNA and RNA synthesis. Magnesium ions are required in hundreds of enzymes and present in every cell type in every organism. Different cell types maintain different concentrations of magnesium [16-17] in animals and plants [18-19]. Magnesium deficiency in the body leads to asthma, diabetes and osteoporosis [20]. Zinc is a versatile ion as it can bind to different combinations of ligand types resulting in a broad range of stability, reactivity and functions. Thousands of proteins contain zinc [21] and the structures of over 200 zinc-containing enzymes have been characterized [22].

Cetyl trimethyl ammonium bromide (CTAB) is a cationic surfactant which tends to denature proteins and profoundly influences the bulk properties of physiological systems. They can solubilise, concentrate and compartmentalize ions and molecules [23]. Cationic micellar media can shift acid-base equilibria. This shift can be explained in terms of differences between the properties of the bulk solvent and of the interfacial region and perturbation of the acid–base equilibria by the electrostatic field effect of the charged interface. The dissociation equilibria of substituted benzoic acids in cationic and anionic micelles have been investigated potentiometrically [24]. It was shown that their pKa values shift to about 0.5–3.0 in anionic micelles. The acid-base equilibria of a number of phenols, amines and carboxylic acids in aqueous micellar solutions have been examined [25]. The present work is an attempt to study the speciation of complexes of Ca (II), Mg (II) and Zn (II) with Histamine in CTAB-water mixtures has been undertaken based on their involvement in various physiological reactions.

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EXPERIMENTAL

Chemicals and Standard Solutions

Histamine (Him) ((Spectrochem),) solution (0.05 mol L^{-1}) was prepared in triple-distilled deionised water by maintaining 0.05 mol L^{-1} hydrochloric acid concentration to increase the solubility. CTAB (Merck, India) was used as received. 2 mol L^{-1} sodium chloride (Qualigens, India) was prepared to maintain the ionic strength in the titrand. 0.1 mol L^{-1} aqueous solutions of Ca (II), Mg (II) and Zn (II) chlorides were prepared by dissolving G.R. Grade (E-Merck, Germany) salts in triple-distilled water maintaining 0.05 mol L^{-1} hydrochloric acid to suppress the hydrolysis of metal salts. All the solutions were standardized 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 [26-31]. The strengths of alkali and mineral acid were determined using the Gran plot method [32, 33].

Instrumentation

The titrimetric data were obtained using Metrohm (Model 905) Auto titrator (readability 0.001), which was calibrated with 0.05 mol L^{-1} potassium hydrogen phthalate in acidic region and 0.01 mol L^{-1} borax solution in basic region. The glass electrode was equilibrated in a well stirred CTAB-water mixture containing the inert electrolyte. All the titrations were carried out in the medium containing varying concentrations of CTAB-water mixtures (0.0-2.5% *v/v*) by maintaining an ionic strength of 0.16 mol $L^{-1}$with sodium chloride at 303.0 ± 0.1 K. The effect of variation in asymmetry potential, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of glass electrode was accounted for in the form of correction factor [34, 35].

Analytical Procedures

For the determination of stability constants of metal-ligand binary species, 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 CTAB-water mixture of equivalent composition as that of titrand. In each of the titrations, the titrand consisted of approximately 1 mmol mineral acid in a total volume of 50 ml. Titrations with different metal to ligand ratios (1:2.5, 1:3.75, 1:5) were carried out with 0.40 mol L^{-1} sodium hydroxide. The analytical concentrations of the ingredients are given in Table 1. Other experimental details are given elsewhere [36-37].

Table 1: Total initial concentrations of ingredients (in mmol) of titrands in CTAB-water mixtures [NaOH] = 0.4 mol L^{-1}; V_0=50.0 cm^3; temp=303 K; ionic strength= 0.16 mol L^{-1}; mineral acid= 1mmol.

% w/v CTAB	TM0			TL0(Him)	TL0:TM0
	Ca(II)	Mg(II)	Zn(II)		
0.0	0.10031	0.10019	0.10012	0.2465 0.3697 0.4932	2.50 3.75 5.00
0.5	0.10031	0.10019	0.10012	0.2475 0.3712 0.4952	2.50 3.75 5.00
1.0	0.10031	0.10019	0.10012	0.2495 0.3742 0.499	2.50 3.75 5.00
1.5	0.10031	0.10019	0.10012	0.2462 0.3697 0.4934	2.50 3.75 5.00
2.0	0.10031	0.10019	0.10012	0.2482 0.3721 0.4967	2.50 3.75 5.00
2.5	0.10031	0.10019	0.10012	0.2505 0.3757 0.5012	2.50 3.75 5.00

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Modeling Strategy

The computer program SCPHD [38-40] was used to calculate the correction factor. By using the pH-metric titration data, the binary stability constants were calculated with the computer program MINIQUAD75 [41], which exploit the advantage of the constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm. During the refinement of binary systems, the correction factor and the protonation constants of histamine were fixed. The variation of stability constants with the mole fraction of the medium was analyzed on electrostatic grounds on the basis of solute-solute and solute-solvent interactions.

RESULTS AND DISCUSSION

Table 2: Parameters of best fit chemical models of Ca (II), Mg (II) and Zn (II) - histamine complexes in CTAB-water mixtures

% w/v CTAB	log β_{mlh} (SD)			pH-Range	NP	U_{corr}	χ^2	Skewness	Kurtosis	R-factor
	MxH	Mx_2	Mx_2H							
Ca(II)										
0.0	10.16(25)	7.73(14)	15.06(20)	2.0-9.0	57	18.93	29.94	2.59	14.95	0.0220
0.5	10.09(25)	7.44(23)	15.20(37)	2.7-8.9	34	15.97	26.00	-1.44	6.82	0.0356
1.0	9.76(38)	7.30(41)	16.11(28)	2.7-9.2	21	13.30	23.97	-0.19	4.37	0.0429
1.5	9.52(32)	7.74(35)	15.41(26)	2.6-8.9	22	16.51	22.33	-1.30	5.89	0.0410
2.0	9.59(22)	8.004(35)	15.38(31)	2.7-7.5	26	21.51	30.31	-0.87	9.63	0.0401
2.5	9.51(31)	8.50(44)	16.07(31)	2.5-7.5	29	19.35	21.78	-1.09	7.16	0.0443
Mg(II)										
0.0	10.05(22)	8.67(28)	17.30(31)	2.4-9.9	52	12.83	7.52	-0.01	8.04	0.0388
0.5	9.89(24)	9.17(38)	17.25(37)	2.8-7.9	37	17.87	16.53	-0.91	11.10	0.0476
1.0	9.60(30)	8.96(36)	16.89(25)	2.4-8.2	36	33.04	22.33	-0.74	4.33	0.0356
1.5	9.87(26)	9.11(28)	16.53(31)	2.8-8.9	25	26.54	33.21	0.43	7.24	0.0355
2.0	9.71(30)	9.23(28)	16.01(26)	2.4-8.9	22	8.76	29.65	4.77	29.05	0.0367
2.5	9.87(32)	8.75(35)	15.63(21)	2.8-8.2	20	10.10	13.73	-0.74	3.97	0.0426
Zn(II)										
0.0	10.54(24)	9.86(32)	16.30(27)	2.5-8.0	48	19.17	11.33	0.49	6.83	0.0336
0.5	9.98(23)	9.28(26)	15.76(23)	2.6-7.9	55	28.34	25.31	1.26	8.18	0.0253
1.0	10.03(27)	9.55(36)	16.43(31)	2.6-8.0	30	16.06	15.69	0.99	5.51	0.0464
1.5	9.95(22)	9.06(35)	16.04(25)	2.5-8.2	40	12.31	21.60	-0.49	5.81	0.0505
2.0	9.81(23)	9.35(36)	16.01(29)	2.6-8.0	54	14.86	20.35	1.19	7.49	0.0307
2.5	9.56(27)	8.93(35)	15.95(22)	2.6-8.3	43	33.28	21.23	-0.21	8.01	0.0413

U_{corr} = U/ (NP-m) X 10^8; NP = Number of points; m = number of protonation constants; SD = Standard deviation

The results of the final best-fit models that contain the stoichiometry of the complex species and their overall formation constants along with some of the important statistical parameters are given in Table 2. Very low-standard deviation in overall stability constants (log *β*) signifies the precision of these constants. The small values of U_{corr} (sum of squares of deviations in concentrations of ingredients at all experimental points) corrected for degrees of freedom, small values of mean, standard deviation and mean deviation for the systems are validated by the residual analysis [42-46].

Residual Analysis

In data analysis with least squares methods, the residuals (the differences between the experimental data and the data simulated based on model parameters) are assumed to follow Gaussian or normal distribution. When the data are fit into the models, the residuals should ideally be equal to zero. If statistical measures of the residuals and the errors assumed in the models are not significantly different from each other, the model is said to be adequate. Further, a model is considered adequate only if the residuals do not show any trend. Respecting the hypothesis that the errors are random, the residuals are tested for normal distribution. Such tests are χ^2, Skewness, Kurtosis and R-factor. These statistical parameters show that the best-fit models portray the metal-ligand species in CTAB-water mixtures, as discussed below.

In the present study, the χ^2 values are less than the table values, and so the models are accepted. The kurtosis values in this study indicate that the residuals form leptokurtic pattern. The values of skewness recorded in Table. 2 are between -1.44 to 2.59 for Ca (II) and -0.91 to 4.77 for Mg (II) and -0.49 to 1.26 for Zn (II). These data evince that the residuals form part of a normal distribution. Hence, least square method can be applied to the present data. The sufficiency of the model is further evident from crystallographic R-values. These statistical parameters thus show that the best-fit models portray the metal-ligand species in CTAB media.

Effect of Systematic Errors on Best Fit Model

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 undertaken by introducing pessimistic errors in the influential parameters like concentrations of alkali, mineral acid, ligand, metal, log F and volume (Table 3). The order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors is alkali > acid > metal > ligand > volume > log *F*. Some species were even rejected when errors were introduced in the concentrations. The rejection of some species and increased standard deviations in the stability constants on introduction of errors confirm the suitability of the experimental conditions (concentrations of ingredients) and choice of the best-fit models.

Table 3: Effect of errors in influential parameters on Zn (II)-histamine complex stability constants in 2.0% *w/v* CTAB-water mixture.

Ingredient	% Error	Log β_{mlh}(SD)		
		111	120	121
Acid	0	9.81(23)	9.35(33)	16.01(28)
	-5	Rejected	10.25(63)	17.18(47)
	-2	9.55(14)	9.91(46)	16.87(33)
	+2	9.92(48)	8.98(35)	16.22(30)
	+5	9.72(57)	3.97(44)	Rejected
Alkali	-5	10.22(35)	5.67(42)	Rejected
	-2	10.00(45)	9.10 (33)	16.44(27)
	+2	9.61(11)	9.79(45)	16.76(33)
	+5	Rejected	10.18(56)	16.87(42)
Ligand	-5	9.96 (32)	9.13(37)	16.30(33)
	-2	9.91(29)	9.39(36)	16.54(30)
	+2	9.70(24)	9.49(38)	16.72(27)
	+5	9.35(15)	9.56(37)	16.79(25)
Metal	-5	9.77(32)	9.41(37)	16.56(29)
	-2	9.86(24)	9.39(37)	16.55(30)
	+2	9.96(19)	9.38(38)	16.52(31)
	+5	10.02(25)	9.34(40)	16.50(33)
Volume	-5	9.83(25)	9.28(36)	16.45(29)
	-2	9.87(23)	9.35(36)	16.50(29)
	+2	9.94(22)	9.45(37)	16.57(30)
	+5	10.00(21)	9.56(38)	16.62(31)
Log F	-5	9.96(24)	9.46(39)	16.60(31)
	-2	9.93(22)	9.41(36)	16.56(30)
	+2	9.89(23)	9.35(36)	16.51(30)
	+5	9.85(31)	9.35(39)	16.46(32)

Effect of Solvent

Many workers were opinion that both electrostatic and non-electrostatic effects should be considered even in the case of simple acido-basic equilibria; one dominates the other, depending upon the

nature of solute and solvent [47-49]. The effect of surfactant on complex equilibria and apparent shift in the magnitude of stability constants in micellar media can be attributed [50] to the creation of a concentration gradient of proton between the interface and the bulk solution. The number of micelles increases with the concentration of surfactant, and oppositely charged ions are concentrated in the Stern layer [51]. The dielectric constant of the medium has a direct influence on the protonation-deprotonation equilibria [52, 53]. According to Born equation, the energy to electrostatic interaction is related to dielectric constant of medium [54]. The variations in log βs of complexes of histamine with mole fraction of CTAB-water mixtures are given in Figure.1. The linear trend indicates that long range interaction between metal ion and ligand is electrostatic in nature. The deviation from linearity may be due to some contributions from non-electrostatic forces.

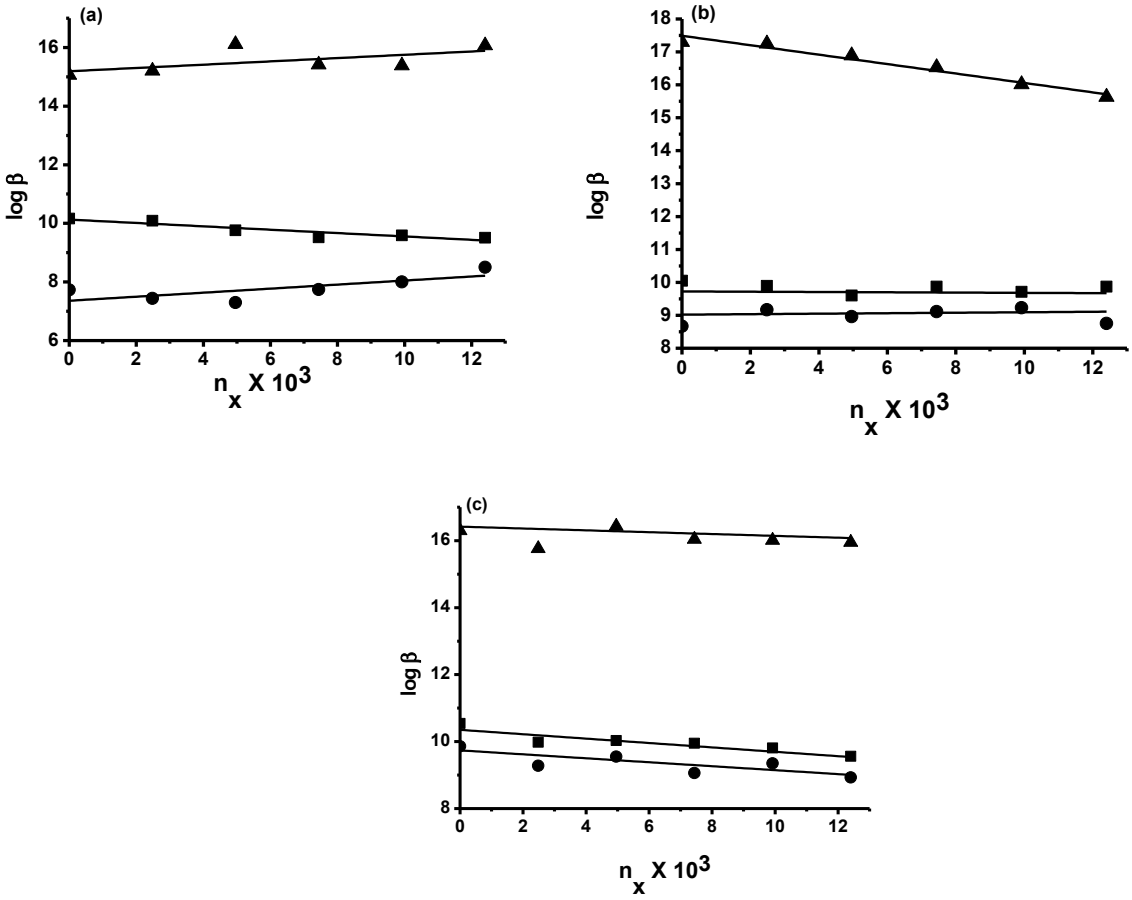

Figure 1: Variation of overall stability constant values of metal-histamine complexes with mole fraction ($n_x \times 10^3$) of CTAB-water mixtures (a) Ca (II); (b) Mg(II); (c) Zn(II); (■)log β_{MXH}, (●) log β_{MX2} ,(▲) log β_{MX2H}.

Distribution Diagrams

Histamine exhibits two main important basic functionalities such as primary aliphatic amine (pKa_1 9.4) and imidazole (pKa_2 5.8). The different forms of histamine are XH_2^{2+}, XH^+, and X^- in the pH ranges viz., below 7.0, 4.5-10.5 and above 9.0, respectively. Hence, the plausible binary metal-ligand species in different systems can be predicted from these data and confirmed by MINIQUAD75. The present investigation reveals the existence of MXH, ML_2 and ML_2H for Ca (II), Mg (II) and Zn (II).

The formation of MX_2H by Ca (II), Mg (II) and Zn (II) probably indicates that the side chain amino group is still protonated in the presence of Ca (II), Mg (II) and Zn (II). The MX_2 species is the predominant species at higher pH and MXH is the predominant species at lower pH among all the binary complexes. Low concentration of free metal ion (FM) indicates the strong complexing nature of histamine. The formation of various binary complex species is shown in the following equilibria. Some typical distribution diagrams of

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CTAB-Water media are shown in Figure. 2. The present investigation reveals the existence of MXH, MX_2H and MX_2 for Ca (II), Mg (II) and Zn (II).

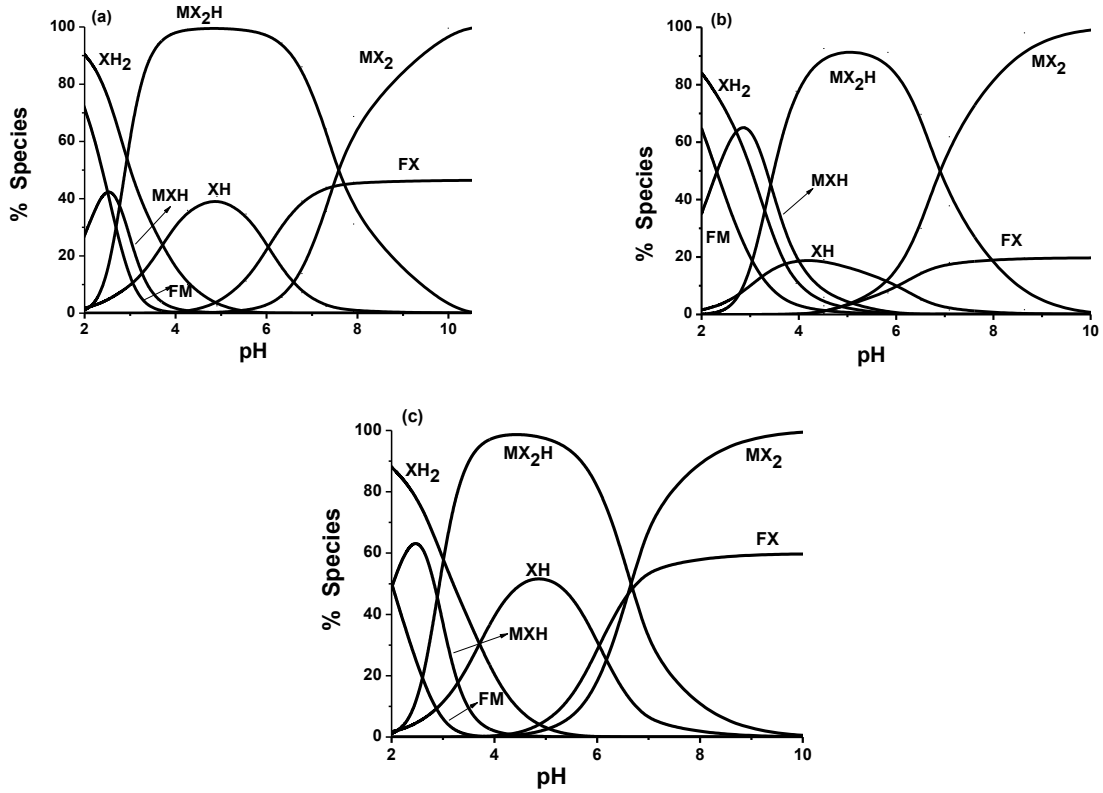

Figure 2: Distribution diagrams of binary complexes of histamine in 1.0% w/v CTAB-water mixture: (a) Ca (II), (b) Mg (II) and (c) Zn (II).

MXH, MX_2H are formed by the reaction of metal with XH_2 [Equilibria (1), and (4)]. Equilibrium (5), (6) and (7) are proposed for formation of MX_2H. MX_2 is also formed by the deprotonation of MX2H [Equilibria (6)]. MXH is formed at lower pH. MX_2H and MX_2 are formed with the increasing pH. MX_2H and MX_2 species percentage successively increases with increasing pH. The concentration of MXH species decreased, while the concentration of MX_2H and MX_2 increased. The formation equilibria can be represented as follows. The charges of the species are omitted for simplicity.

$M(II) + XH_2^{2+}$	⇌	$MXH^{3+} + H^+$	(1)
MXH^{3+}	⇌	$MX^{2+} + H^+$	(2)
$M(II) + XH_2^{2+}$	⇌	$MX^{2+} + 2H^+$	(3)
$M(II)+2XH_2^{2+}$	⇌	$MX_2H_2^{4+} +2H^+$	(4)
$MX_2H_2^{4+}$	⇌	$MX_2H^{3+} + H^+$	(5)
$M(II) +2XH_2^{2+}$	⇌	$MX_2H^{3+} + 3H^+$	(6)
$MX^{2+} + LH_2^{2+}$	⇌	$MX_2H^{3+}+ H^+$	(7)
MX_2H^{3+}	⇌	$MX_2^{2+} + 2H^+$	(8)
$MX^{2+}+ XH_2^{2+}$	⇌	$MX_2^{2+} + 2H^+$	(9)

Structures of Complexes

Histamine contains three functional groups: a) the a-amino group, b) the secondary imidazole nitrogen, and c) the tertiary imidazole nitrogen. However, it has been shown that the secondary nitrogen atom does not take part in the coordination reaction. Histamine therefore acts as a bidentate ligand, when coordinating to metal ion via the nitrogen atoms of the amino and imidazol groups.This additional chelation results in a five-membered ring, which is proved from the X-ray diffraction analysis of Histamine complexes of transition metal ions (Co, Cu, Zn and Cd) [55, 56]. Octahedral structures are proposed to the complexes of all

the metal ions. The VSEPR theory suggests that Ca (II), Mg (II) and Zn (II) complexes shall be octahedral because there are six outer electron pairs. Amino nitrogen atom histamines can associate with hydrogen ions in physiological pH ranges. Hence, there is often significant competition between hydrogen and metal ion for this second donor site. This situation results in the simultaneous existence of a number of equilibria producing an array of successively protonated complexes. Hence, protonated complex species are detected in the present study. This argument supports the structures of complexes proposed in Figure 3.

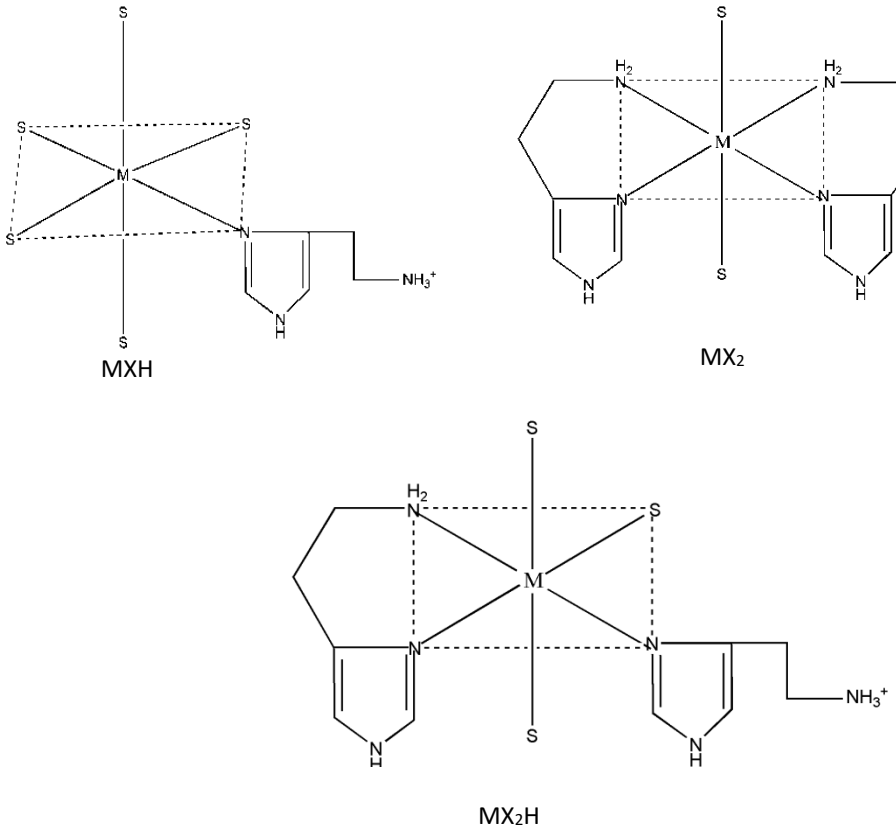

Figure 3: Structure of histamine complexes (S is either solvent or water molecules).

CONCLUSIONS

The following conclusions have been drawn from the modeling studies of the speciation of binary complexes of Ca (II), Mg (II) and Zn (II) with histamine in CTAB-water mixture.

- Forms both protonated and unprotonated complexes under a pH range of 2.0-10.0
- The binary species formed due to the interaction of histamine with metals are CaXH, CaX_2, CaX_2H, MgXH, MgX_2, MgX_2H, ZnXH, ZnX_2 and ZnX_2H. These models are validated by statistical treatment of data.
- The linear variation of stability constants as a function of dielectric constant of the medium indicates the dominance of electrostatic forces over non-electrostatic forces. Some species are stabilized due to electrostatic interactions and some are destabilized due to the decreased dielectric constant.
- The order of ingredients influencing the magnitudes of stability constants due to incorporation of errors in their concentrations is alkali > acid > ligand > metal > total volume > Log F.

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