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EFFECT OF DIELECTRIC CONSTANT OF MEDIUM ON CHEMICAL SPECIATION OF L-HISTIDINE COMPLEXES OF Co(II), Ni(II) AND Cu(II)

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ABSTRACT. Chemical speciation of binary complexes of Co(II), Ni(II) and Cu(II) ions with L-histidine have been studied pH metrically in the concentration range of 0–60% v/v DMSO-water mixtures maintaining an ionic strength of 0.16 M at 303 K. Alkalimetric titrations were carried out in different relative concentrations of metal and histidine. Stability constants of various models of binary complexes were refined with MINIQUAD75. The best-fit chemical models were selected based on statistical parameters and residual analysis. The species detected are MLH, ML_2, ML_2H, ML_2H_2 and ML_2H_4 for Co(II); ML_2, ML_2H, ML_2H_2 and ML_2H_4 for Ni(II); and MLH, ML_2, ML_2H and ML_2H_2 for Cu(II). The chemical speciation, metal bioavailability and transportation are explained based on the distribution diagrams.

KEY WORDS: L-Histidine, Chemical speciation, Essential metals, Binary complexes, Bioavailability, DMSO

INTRODUCTION

Classical curve-fitting methods that use the least-squares methods [1, 2] are applied to estimate the number of species simultaneously present at equilibrium, their stoichiometries, and their stability constants. Bioavailability of a particular metal depends on its complex chemical reactions of dissolution, binding and complexation with the constituents of the environmental aquatic phase [3]. The metal complexes can be more active than the free ligands and some side effects may decrease upon complexation. In addition, the complexes can exhibit bioactivities which are not shown by the free ligand. The mechanism of action can involve binding to a metal ion in vivo or the metal complex may be a vehicle for activation of the ligand as the cytotoxic agent. Moreover, coordination may lead to significant reduction of drug-resistance [4, 5].

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 behavior 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.

L-Histidine (His) is an essential component of almost all the proteins. It provides metal binding sites in many enzymes. Due to the high reactivity of its imidazole group, histidine residue is often found at the active site of enzymes and involved directly in catalysis. It controls the transmission of metals in biological bases [6]. His containing antibacterial peptides have been reported in few peptides including histatin, clavanin and chrysophsin [7-12]. The formation and structure of histidine complexes with some transition metal ions have been studied in aqueous medium [13-19]. Speciation of the gold(III)-histidine complexes reveals important speciation information for both environmental and medical issues [20].

Cobalt is essential for the production of red blood cells. It acts as coenzyme in several biochemical processes. Cobalt is present in glutamate mutase, dialdehydase, methionine synthase, and arginase. It is present in non-corrin form in dipeptidase. The peptide-Ni

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complexes have higher toxicity than the pure peptides. The glycine- and histidine-containing peptides may carry metal ions into the cells, where they can be released by the amino acid residues with high affinity for heavy metal ions. High flexibility of these peptides allows them free penetration through the membrane in the cytoplasm [21]. Active site of urease has two nickel atoms which are required for its activity. Nickel is also present [22] in carbon monoxide dehydrogenase, methyl-S-coenzyme-M-reductase and hydrogenase. Copper is one of the transition elements frequently found at the active site of proteins. In nature, a wide variety of copper proteins are essential constituents of aerobic organisms, including hemocyanins and enzymes.

The aim of the present study is to understand the role of metal ions at active site cavities in bioactive molecules like enzymes and proteins to know the effect of dielectric constant of the medium on the chemical speciation of the title systems. Histidine has been taken as a model compound for amino acid residues. Since the dielectric constant at the active site cavities is very small compared to that at bio-fluids, low dielectric constant is mimicked by using a water soluble organic solvent like dimethyl sulfoxide (DMSO). DMSO is a polar aprotic solvent. DMSO has a very strong affinity for water.

EXPERIMENTAL

Chemicals

DMSO (Merck, Mumbai) was used as received. Aqueous solutions of L-histidine and sodium chloride (E-Merck, Germany) were prepared in triple distilled water. Metal solutions of Co(II), Ni(II) and Cu(II) chlorides were prepared. To increase the solubility of His and to suppress the hydrolysis of metal salts, the mineral acid concentration in the above solutions was maintained at 0.05 M. 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). The strength (concentration) of alkali was determined using the Gran plot method [23, 24].

Apparatus

The titrimetric data were obtained with a calibrated ELICO (Model L1-120) pH-meter (readability 0.01) which can monitor the changes in H^+ concentration. The pH meter was calibrated with 0.05 M potassium hydrogen phthalate in acidic region and 0.01 M borax solution in alkaline region. The glass electrode was equilibrated in a well-stirred DMSO-water mixture containing inert electrolyte. All the titrations were carried out in the medium containing varying concentrations of DMSO (0-60% v/v) maintaining an ionic strength of 0.16 M with sodium chloride at 303.0 ± 0.1 K. The effect of variations in asymmetry potential, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of glass electrode were accounted for in the form of correction factor [25].

The emf of the cell may be expressed by the equation $E = K + (RT/F) \ln a_{H^+}$ or $E = K + 0.0591$ pH at 25 ^{0}C, where K is a constant partly dependent upon the nature of the glass used for making the membrane. The value of K may vary slightly with time, and it is related to the existence of an asymmetry potential [26] in a glass electrode. Owing to the asymmetry potential, if a glass electrode is inserted into a test solution which is identical with the internal hydrochloric acid solution, the electrode shall have a small potential which is found to vary with time. Hence, glass electrode is standardized frequently using a buffer of known hydrogen activity.

Procedure

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 DMSO-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 ratios (1:2.5, 1:3.5, 1:5.0) of metal-to-ligand were carried out with 0.4 M sodium hydroxide. Other experimental details are given elsewhere [27].

Modeling strategy

The computer program SCPHD [28] was used to calculate the correction factor. The binary stability constants were calculated with the computer program MINIQUAD75 [29] from the pH-metric titration data. The correction factor and the protonation constants of histidine were fixed during the refinement of binary systems. During the modelling study, as the number of species increased, the models gave better statistics denoting the better fit. This indicates that the final models appropriately fit the experimental data. The variation of stability constants with the dielectric constant of the medium was analyzed on electrostatic grounds and on the basis of solute-solute and solute-solvent interactions.

RESULTS AND DISCUSSION

Exhaustive modelling

Existence of various species was determined by performing exhaustive modelling [30] and the results of a typical system are given in Table 1. The models were evaluated assuming the simultaneous existence of different combinations of species. Models containing various numbers and combinations of species were generated using an expert system package CEES [31] and they were refined using MINIQUAD75. As the number of species increased, the models gave better statistics denoting the better fit. This indicates that the final model appropriately fits the experimental data. Such exhaustive modelling is performed on Co(II)-histidine system in 10% DMSO. The table contains the stoichiometric coefficients and stability constants of the complex species, standard deviations in the stability constants and residual statistics of the models.

Model validation

(a) Retrieval of protonation constants

Protonation constants were retrieved from the metal-ligand titrations and compared with those obtained from proton-ligand titration data. The proximity of the two values confirms the existence of only reported metal-ligand species and accuracy of the titration data. Such comparisons for some typical systems are given in Table 2. Then simultaneous refinement of all the constants revealed that when the approximate constants are very close to the true values, either fixing some of the species or ingredient concentrations do not have any ill-effects on modelling studies.

Table 1. Exhaustive modelling study performed on Co(II)-histidine system in 10% DMSO in 4.0-8.0 pH range (number of experimental points, NP = 45).

Model	log βmlh (SD)					U_{corr}	χ^2	Skew	Kurto	R –factor
no	111	120	121	122	124			-ness	-sis	
1	14.19(15)	----	----	----	----	179.77	46.63	-0.46	3.38	0.1038
2	----	13.35(10)	----	----	----	13.36	20.91	-1.08	2.71	0.0283
3	----	----	19.54(13)	----	----	17.14	27.43	0.86	5.65	0.0320
4	----	----	----	26.02(65)	----	126.14	35.37	-0.27	4.40	0.0869
5	----	----	----	----	Rejected	----	----	----	----	----
6	13.25(16)	13.73(15)	----	----	----	3.63	14.63	-0.73	2.45	0.0146
7	12.45(51)	----	19.68(19)	----	----	16.93	59.90	0.96	6.22	0.0315
8	Rejected	----	----	26.02(65)	----	129.07	35.37	-0.27	4.40	0.0869
9	Rejected	----	----	----	Rejected	----	----	----	----	----
10	----	12.77(10)	19.34(7)	----	----	2.84	2.30	-0.75	3.25	0.0129
11	----	13.26(5)	----	25.33(5)	----	1.11	11.07	-0.53	3.65	0.0081
12	----	13.35(10)	----	----	Rejected	13.67	20.91	-1.08	2.71	0.0283
13	----	----	19.55(11)	24.07(15)	----	17.49	25.65	0.88	5.73	0.0320
14	----	----	19.55(17)	----	33.99(410)	17.53	24.59	0.85	5.63	0.0320
15	----	----	----	Rejected	Rejected	----	----	----	----	----
16	12.88(5)	13.19(5)	19.53(4)	----	----	0.37	2.07	0.62	3.88	0.0046
17	Rejected	13.26(5)	----	25.33(5)	----	1.14	11.07	-0.53	3.65	0.0081
18	13.39(27)	13.86(25)	----	----	35.08(100)	3.71	12.26	-0.74	2.50	0.0146
19	12.45(51)	----	19.68(19)	Rejected	----	16.93	59.90	0.96	6.22	0.0315
20	13.50(16)	----	20.62(15)	----	36.56(18)	16.79	39.99	0.96	6.21	0.0310
21	Rejected	----	----	Rejected	Rejected	----	----	----	----	----
22	----	13.04(4)	19.12(60)	25.12(5)	----	0.54	33.83	-0.33	4.05	0.0055
23	----	12.77(10)	19.34(7)	----	Rejected	2.84	2.30	-0.75	3.25	0.0129
24	----	13.60(11)	----	25.77(13)	35.81(22)	0.77	5.98	-1.02	4.55	0.0066
25	----	----	19.75(32)	24.68(100)	35.36(93)	17.83	26.96	0.86	5.62	0.0319
26	12.67(10)	13.16(4)	19.40(6)	24.82(13)	----	0.30	24.59	0.54	4.43	0.0041
27	13.06(11)	13.34(10)	19.69(9)	----	35.16(31)	91.22	2.54	0.78	4.34	0.0043
28	13.50(16)	----	20.62(150)	Rejected	36.56(18)	16.79	39.99	0.96	6.21	0.0310
29	----	13.26(8)	19.24(8)	25.42(10)	35.36(21)	0.44	7.28	-0.86	4.80	0.0050
30	12.15(71)	13.65(15)	----	25.80(15)	35.87(25)	0.78	8.11	-1.02	4.60	0.0066
31	12.89(11)	13.41(8)	19.59(8)	25.25(11)	35.50(17)	0.20	32.53	0.33	7.74	0.0033

Table 2. Retrieval of protonation constants from metal-ligand titration data.

DMSO % v/v	From proton-ligand titration data			From Co(II)-His titration data		
	$\log \beta_1$	$\log \beta_2$	$\log \beta_3$	$\log \beta_1$	$\log \beta_2$	$\log \beta_3$
00	9.26	15.40	17.14	9.26	15.39	17.09
10	9.42	15.57	17.43	9.37	15.53	17.43
20	9.58	15.70	17.69	9.51	15.48	17.73
30	9.66	15.76	17.82	9.72	15.79	18.16
40	9.63	15.60	17.91	9.49	15.45	18.27
50	9.63	15.45	17.94	9.71	15.45	18.60
60	9.70	15.33	18.08	9.81	15.37	18.50

(b) Introduction of pessimistic errors

In order to rely upon the best chemical model for critical evaluation and application under varied experimental conditions with different accuracies of data acquisition, an investigation was made by introducing pessimistic errors in the influential parameters [32] like concentrations of alkali, mineral acid, ligand and metal (Table 3). The order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors is alkali > acid > ligand > metal. Some species are even rejected when errors are introduced in the concentrations. This study confirms the appropriateness of the chosen best-fit models. This study also indicates the relative sensitivities of model parameters.

Table 3. Effect of errors in influential parameters on cobalt-histidine stability constants in 20% v/v DMSO-water mixture.

Ingredient	% Error	log β_{mlh} (SD)				
		MLH	ML_2	ML_2H	ML_2H_2	ML_2H_4
	0	13.19(13)	14.13(9)	20.26(9)	26.03(11)	36.11(15)
Alkali	-5	Rejected	11.56(16)	19.32(12)	25.48(16)	36.24(21)
	-2	12.81(12)	13.25(7)	19.93(6)	25.69(8)	36.09(11)
	+2	13.89(44)	14.95(42)	20.89(4)	Rejected	35.85(84)
	+5	18.25(310)	19.90(310)	25.22(311)	Rejected	Rejected
Acid	-5	18.76(284)	20.52(284)	25.97(285)	Rejected	Rejected
	-2	13.72(18	14.80(17)	20.76(15)	Rejected	Rejected
	+2	12.72(15)	13.34(7)	19.94(7)	25.82(8)	36.31(11)
	+5	Rejected	12.10(28)	19.59(24)	25.78(29)	36.79(33)
Ligand	-5	13.17(16)	14.24(11)	20.34(11)	26.39(11)	36.78(13)
	-2	13.18(14)	14.16(9)	20.28(10)	26.16(11)	36.38(14)
	+2	13.46(15)	14.11(13)	20.43(13)	Rejected	35.50(38)
	+5	13.42(7)	14.15(7)	20.45(5)	Rejected	Rejected
Metal	-5	13.48(20)	14.23(18)	20.45(18)	Rejected	35.73(41)
	-2	13.48(17)	14.13(16)	20.44(16)	Rejected	35.78(34)
	+2	13.21(11)	14.07(8)	20.28(8)	26.02(9)	36.14(13)
	+5	13.24(9)	13.99(7)	20.29(7)	26.01(8)	36.18(11)
log F	-5	13.18(13)	14.10(9)	20.25(9)	26.03(10)	36.14(15)
	-2	13.18(13)	14.12(9)	20.26(9)	26.03(11)	36.12(15)
	+2	13.19(13)	14.14(9)	20.27(9)	26.03(11)	36.10(15)
	+5	13.19(13)	14.16(9)	20.28(10)	26.03(11)	36.08(15)
Volume	-5	13.16(13)	14.09(9)	20.22(9)	25.99(11)	36.07(15)
	-2	13.18(13)	14.11(9)	20.25(9	26.02(11	36.10(15)
	+2	13.19(13)	14.15(9)	20.28(9)	26.05(11)	36.13(15)
	+5	13.21(13)	14.17(9)	20.31(9)	26.07(11)	36.15(15)

Best fit models

The results of the best-fit models that contain the type of species and overall formation constants along with some of the important statistical parameters are given in Table 4. A very low standard deviation in log β values indicates the precision of these parameters. Small values of Ucorr (sum of squares of deviations in concentrations of ingredients at all experimental points corrected for degrees of freedom) indicate that the experimental data can be represented by the models. Small values of mean, standard deviation and mean deviation for the systems corroborate that the residuals are around a zero mean with little dispersion. For an ideal normal

distribution, the values of kurtosis and skewness should be three and zero, respectively. Kurtosis is a measure of the peakedness of the error distribution near a modal value. For an ideal normal distribution kurtosis value should be three (mesokurtic). If the 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 as well as platykurtic patterns and very few are nearer to mesokurtic patterns. The values of skewness recorded in Table 4 are between -0.67 and 1.34. These data evince that the residuals form part of a 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-value recorded. These statistical parameters thus show that the best-fit models portray the metal-ligand species in DMSO-water mixtures.

Table 4. Best-fit chemical models of histidine complexes of Co(II), Ni(II) and Cu(II) in DMSO-water mixtures (temperature = 303 K, ionic strength = 0.16 M).

DMSO	log β (SD)					pH-	NP	U_{corr}	Skew-	Kurt-	χ^2	R-factor
% v/v	MLH	ML_2	ML_2H	ML_2H_2	ML_2H_4	range			ness	osis		
Co(II)												
00.0	12.68(20)	12.83(14)	19.09(14)	24.91(19)	35.35(26)	4.0-8.0	45	0.60	0.47	3.86	7.28	0.0058
10.0	12.89(11)	13.41(8)	19.59(8)	25.25(11)	35.50(17)	4.0-8.0	45	0.20	0.33	7.74	32.53	0.0033
20.0	13.19(13)	14.13(9)	20.26(9)	26.03(11)	36.11(15)	4.0-8.0	44	0.20	1.20	7.60	26.06	0.0034
30.0	12.82(26)	14.01(12)	20.41(11)	26.00(15)	35.79(27)	4.0-8.0	43	0.61	0.22	2.64	2.53	0.0057
40.0	12.56(54)	14.61(15)	20.34(18)	26.08(18)	35.43(37)	4.0-7.0	46	1.17	0.34	4.51	21.36	0.0078
50.0	13.02(23)	14.72(12)	21.02(12)	26.37(14)	35.83(18)	4.2-8.0	45	0.37	-0.49	3.99	14.16	0.0045
60.0	13.66(54)	15.69(46)	21.92(44)	26.58(62)	35.75(62)	4.0-8.0	43	3.58	-0.14	3.27	9.60	0.0138
Ni(II)												
00.0	----	16.13(7)	21.38(12)	26.99(6)	35.39(8)	2.0-7.0	93	0.80	-0.28	4.78	26.75	0.0047
10.0	----	16.65(6)	21.73(12)	27.29(5)	35.25(8)	2.0-7.0	95	0.65	-.0.25	4.42	33.79	0.0041
20.0	----	16.94(12)	22.32(11)	27.62(12)	35.59(29)	2.7-7.0	58	1.15	1.34	8.43	44.11	0.0071
30.0	----	17.87(24)	23.04(23)	28.44(24)	36.66(32)	3.2-7.0	46	1.27	0.53	3.64	1.83	0.0079
40.0	----	18.75(8)	22.98(14)	28.08(5)	35.05(15)	2.2-7.0	85	1.18	-0.67	5.54	17.09	0.0059
50.0	----	18.98(11)	23.61(13)	28.41(10)	35.40(21)	3.0-6.0	49	0.84	0.22	3.90	8.48	0.0060
60.0	----	19.64(9)	24.04(12)	28.68(8)	35.34(18)	3.1-7.0	53	0.60	0.05	3.48	5.68	0.0053
Cu(II)												
00.0	14.23(7)	17.97(6)	24.03(4)	28.21(5)	-----	2.8-7.5	52	0.35	-0.09	3.75	10.77	0.0042
10.0	14.57(4)	18.44(4)	24.58(3)	28.53(4)	-----	2.8-7.5	51	0.17	-0.29	3.80	3.39	0.0029
20.0	14.91(4)	19.29(5)	25.27(3)	29.05(5)	-----	2.8-7.5	52	0.21	-0.33	3.89	6.56	0.0032
30.0	14.56(6)	18.41(5)	24.87(4)	29.14(3)	-----	2.8-7.5	49	0.26	-0.34	4.01	11.42	0.0035
40.0	14.70(6)	18.58(7)	24.97(5)	29.02(5)	-----	2.8-7.5	57	0.49	0.50	4.44	9.91	0.0047
50.0	15.11(6)	19.50(7)	25.48(6)	29.41(4)	-----	2.8-7.5	55	0.39	-0.45	4.05	3.87	0.0043
60.0	15.63(5)	20.41(5)	26.18(5)	30.15(3)	-----	2.8-7.5	54	0.11	-0.58	7.80	33.36	0.0022

Ucorr = U/(NP-m) x 10^8, where, m = number of species; NP = number of experimental points; SD = standard deviation.

Influence of dielectric constant

The water-DMSO mixture influences microscopic dynamics of solvated ions [33, 34] and dye molecules [35, 36] so that these solutes exhibit a qualitatively different behavior compared to the behavior in other solvents. The variation of overall stability constant values or change in free energy with co-solvent content depends upon two factors, viz., electrostatic and non-electrostatic. Born's [37] classical treatment holds good in accounting for the electrostatic contribution to the free energy change. According to this treatment, the energy of electrostatic interaction is related to dielectric constant. Hence, the log β values should vary linearly as a

function of the reciprocal of the dielectric constant (1/D) of the medium, which is observed in the present study (Figure 1). The linear variation indicates that electrostatic forces dominate the equilibrium process under the present experimental conditions. It also indicates that the dielectric constant or long range interactions are responsible for the stability trend.

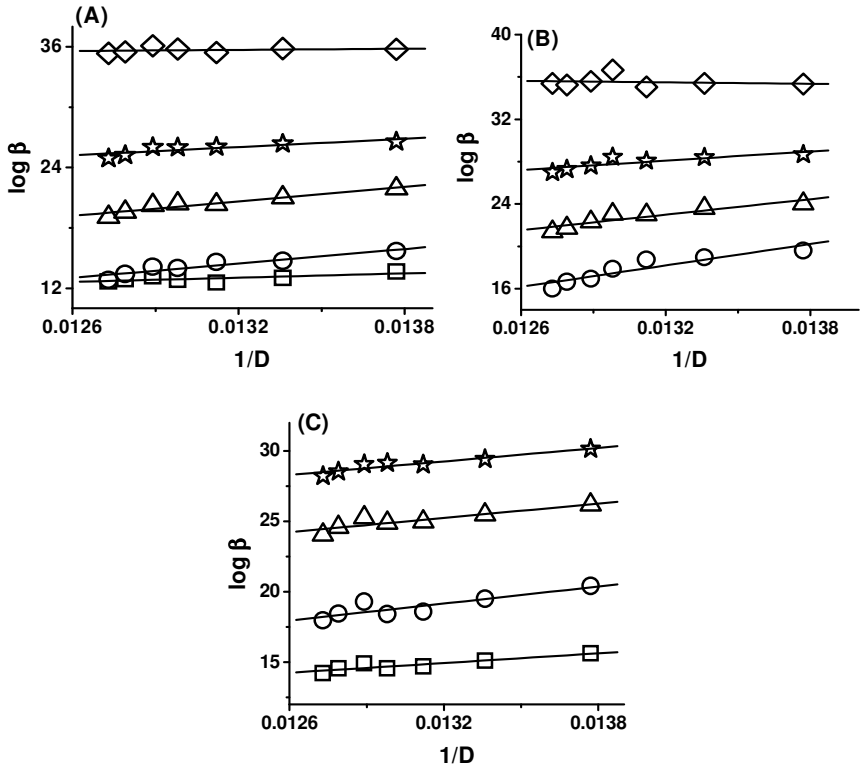

Figure 1. Variation of stability constant values of metal-histidine complexes with reciprocal of dielectric constant in DMSO-water mixtures at temperature = 303 K, ionic strength = 0.16 M. (A) Co(II), (B) Ni(II) and (C) Cu(II) ; (□) log β_{111}, (o) log β_{120} (Δ) log β_{121}, (☆) log β_{122} (◊) log β_{124}.

Distribution diagrams

His has one dissociable carboxyl proton and its amino and imidazole groups can associate with one proton each. The different forms of His are LH_3^{+2}, LH_2^+ and LH in the pH regions 1.5-3.0, 2.0-7.0 and 5.0-9.0, respectively. Hence, the plausible species in different systems can be predicted from these data. The species refined and determined are MLH, ML_2, ML_2H, ML_2H_2 and ML_2H_4 for Co(II) ; ML_2, ML_2H, ML_2H_2 and ML_2H_4 for Ni(II) ; MLH, ML_2, ML_2H and ML_2H_2 for Cu(II) in the pH ranges 3.0-8.0, 2.0-7.0 and 3.0-8.0, respectively. The formation of various binary complex species is shown in the following equilibria. The charges of species are omitted for simplicity.

$$M(II) + 2LH_3 \rightleftharpoons ML_2H_4 + 2H^+ \quad (1)$$

$$ML_2H_4 \rightleftharpoons ML_2H_3 + H^+ \quad \text{(minor process)} \quad (2)$$

$$ML_2H_3 \rightleftharpoons ML_2H_2 + H^+ \quad (3)$$

$$M(II) + 2LH_3 \rightleftharpoons ML_2H_2 + 4H^+ \quad (4)$$

$$MLH + LH_2 \rightleftharpoons ML_2H_2 + H^+ \quad (5)$$

$$ML_2H_2 \rightleftharpoons ML_2H + H^+ \quad (6)$$

$$M(II) + LH_3 \rightleftharpoons MLH + 2H^+ \quad (7)$$

$$M(II) + LH_2 \rightleftharpoons MLH + H^+ \quad (8)$$

$$M(II) + 2LH_2 \rightleftharpoons ML_2H_2 + 2H^+ \quad (9)$$

$$M(II) + 2LH_2 \rightleftharpoons ML_2H + 3H^+ \quad (10)$$

$$M(II) + 2LH_2 \rightleftharpoons ML_2 + 4H^+ \quad (11)$$

$$M(II) + 2\,LH \rightleftharpoons ML_2 + 2H^+ \quad (12)$$

$$ML_2H \rightleftharpoons ML_2 + H^+ \quad (13)$$

Some typical distribution diagrams in DMSO-water mixtures are shown in Figure 2 which indicate the formation of binary complexes of Co(II), Ni(II) and Cu(II). At a pH below 3.0 ML_2H_4 species is formed for Co(II) and Ni(II) [Equilibrium 1]. This might have been deprotonated to ML_2H_3 which might have been quickly deprotonated to ML_2H_2 above a pH of 3.0 [Equilibria 2 and 3]. ML_2H_3 could not be detected probably due its instability or it may be a transient species. Simultaneous increase in the concentrations of ML_2H_2 and MLH supports Equilibria 3, 5 and 8. Simultaneous formation of ML_2H_2 and ML_2H supports Equilibria 9 and 10. ML_2H_2 deprotonates to ML_2H with increasing pH [Equilibrium 6]. ML_2 is formed in the order of Equilibria 11-13 with increasing pH. In the case of Cu-His complexes (Figure 2C) concentrations of LH_3 and free metal ion decrease with increasing concentration of MLH species [Equilibrium 7]. Formation of ML_2H_2 can be explained from Equilibria 4, 5 and 9. Successive deprotonation of ML_2H_2 beyond a pH of 4.0 forms ML_2H and ML_2 [Equilibria 6 and 13].

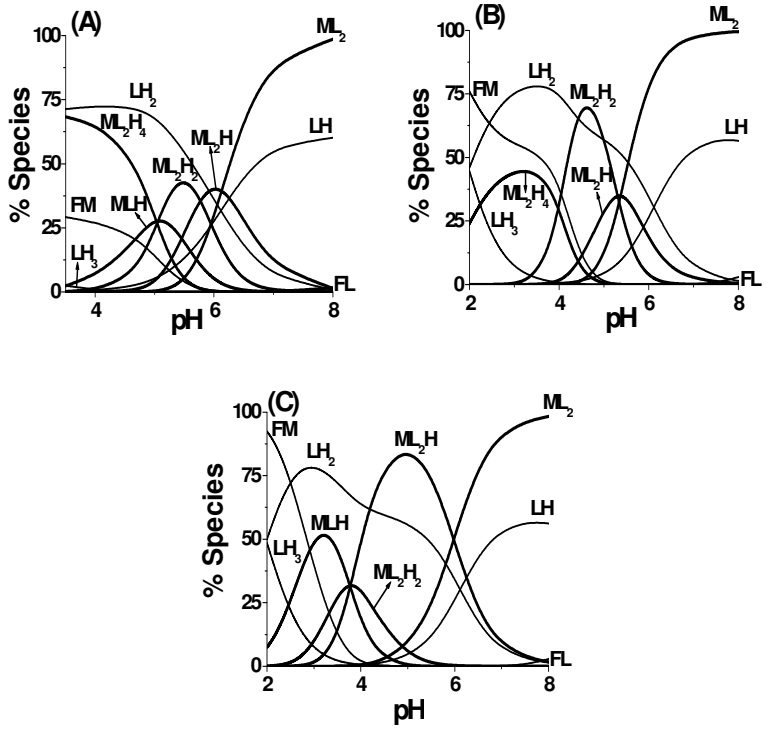

Figure 2. Distribution diagrams of histidine complexes in 20% v/v DMSO-water mixture at 303 K and 0.16 M ionic strength. FM and FL are the free metal and the most deprotonated form of the ligand, respectively. (A) Co(II) = 0.0935 mmol, (B) Ni(II) = 0.0978 mmol and (C) Cu(II) = 0.099 mmol. In all the systems the number of mmols of His is 0.50.

Structures of complexes

When the second donor site of His is a nitrogen atom, marked bidentate behavior is frequently found, more so when the additional chelation results in a five- and seven-membered rings (Figure 3). Octahedral structures are proposed to the complexes of all the metal ions. Amino and imidazole nitrogen atoms of histidine can associate with hydrogen ions in physiological pH ranges. Hence, there is often significant competition between hydrogen and metal ion for these donor sites. 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. All the complexes were protonated in acidic pH region (up to pH 5). Under these conditions, the imidazole and α-amino nitrogen atoms of hisitidine are protonated and carboxyl group is bonded to the metal ion. As the pH increases, the nitrogen atoms are deprotonated and coordinated to the metals.

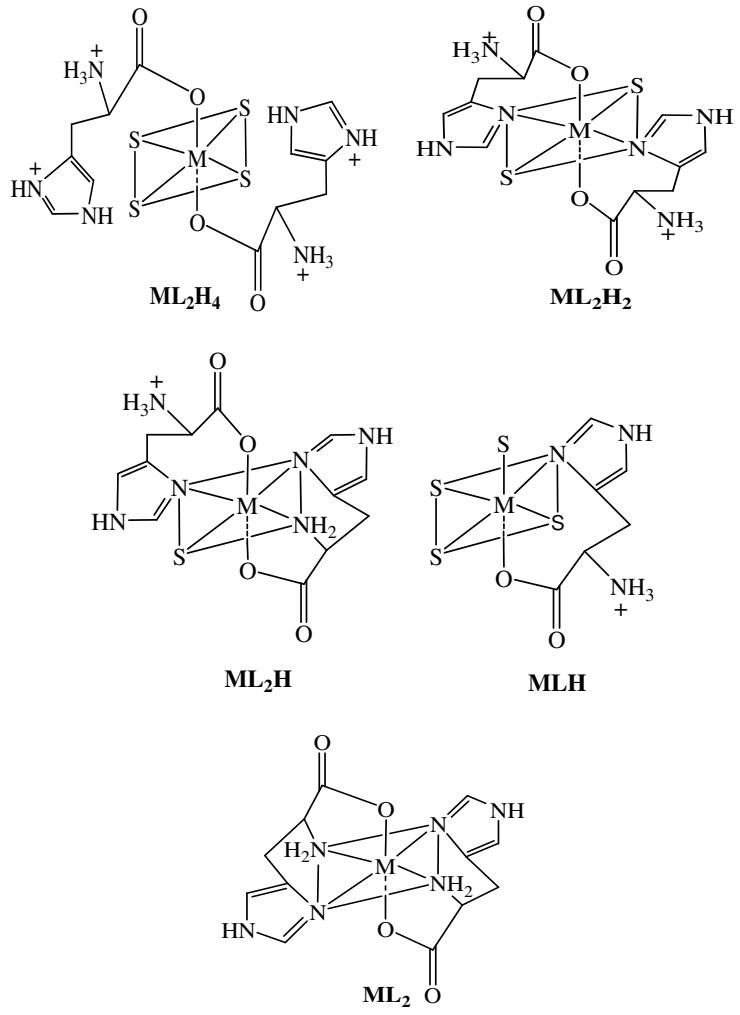

Figure 3. Structures of L-histidine complexes, where S is either solvent or water molecules.

CONCLUSIONS

The present biomimetic studies of metal ion complexes with L-histidine in DMSO-water mixtures indicate the formation of protonated complexes in acidic pH (up to pH 5). Under these pH conditions, the imidazole and α-amino nitrogen atoms of hisitidine are protonated and carboxyl group is bonded to the metal ion. As the pH increases, the nitrogen atoms are deprotonated and coordinated to the metals. The species formed due to the interaction of L-histidine with the metals are MLH, ML, ML_2H_4, ML_2H_2, ML_2H and ML_2. The linear increase in log β values with 1/D of the medium indicates the dominance of electrostatic forces over non-electrostatic forces. The influence of errors in the concentrations of ingredients on the

magnitudes of stability constants is alkali > acid > ligand > metal. Proximity of the protonation constants determined from proton ligand titration data to those retrieved from metal ligand titration data confirm the sufficiency of the models. High concentrations of the complex chemical species indicate that the metals are more amenable for transportation at biological pH.

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