Co^{II},Ni^{II}和 Cu^{II}与 L-组氨酸和 L-谷氨酸在 1,4 二氧六环–水混合物中混合配体配合物的生成平衡

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摘要：在 303.0 K 维持离子强度为 0.16 mol·L^{-1},体积比为 0.0~60.0% 的 1,4 二氧六环–水混合体系中用 pH 滴定法研究了 Co^{II},Ni^{II}和 Cu^{III}离子与 L-组氨酸和 L-谷氨酸混合配体配合物的生成平衡。对不同的金属(M)与 L-组氨酸(L)、L-谷氨酸(X)比(c_M:c_L:c_X=1:2.5:2.5,1:2.5:5.0,1:5.0:2.5)的体系进行了碱量滴定。计算了三元配合物的稳定常数并用 MINIQUAD75 对各种模型进行了优化。根据统计数据和残差分析结果选取了最合适的化学模型。检测到的物种：对 Co^{II}而言为 MLX$_2$H^{2-}和 MLX$_2$$^{3-}$,对 Ni^{II}和 Cu^{II}而言为 MLXH,MLX$_2$H^{2-}和 MLX$_2$$^{3-}$。就有关浓度和稳定性对三元物种与二元物种进行了比较。三元物种的额外稳定性可归因于电荷中和,螯合效应,堆叠作用和氢键等。

关键词：化学物种；混合配体配合物；氨基酸；组氨酸；谷氨酸
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Mixed-Ligand Complex Formation Equilibria of Co^{II}, Ni^{II} and Cu^{II} with L-Histidine and L-Glutamic Acid in Dioxane-Water Mixtures

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Abstract: Mixed-ligand complex formation of Co^{II}, Ni^{II} and Cu^{II} ions with L-histidine and L-glutamic acid was studied pH metrically in 0.0~60.0% (V/V) dioxane-water mixtures maintaining an ionic strength of 0.16 mol·L^{-1} at 303.0 K. Alkalimetric titrations were carried out in different relative concentrations (c_M:c_L:c_X=1:2.5:2.5, 1:2.5:5.0, 1:5.0:2.5) of metal (M) to histidine (L) to glutamic acid (X). Stability constants of ternary complexes were calculated and various models were refined with MINIQUAD75. The best fit chemical models were selected based on statistical parameters and residual analysis. The species detected are MLX$_2$H^{2-} and MLX$_2$$^{3-}$ for Co^{II}, and MLXH, MLX$_2$H^{2-} and MLX$_2$$^{3-}$ for Ni^{II} and Cu^{II}. The relative concentrations and stabilities of the ternary species are compared with those of binary species. The extra stability associated with the ternary complexes is attributed to factors such as charge neutralization, chelate effect, stacking interactions and hydrogen bonding.

Key words: chemical speciation; mixed-ligand complexes; amino acids; histidine, glutamic acid

0 Introduction

Mixed ligand complexes play a significant role in biological systems[1]. Synthesis and structural studies of mixed ligand transition metal complexes with histidine and adenine or guanine[2] and other amino acids[3-6] have been reported. Cobalt is an essential metal widely distributed in the biological systems such

as cells and body, and thus the interaction of DNA with cobalt complex has attracted much attention [7]. The binding properties of cobalt with calf thymus DNA show that the size and shape of the intercalated ligand has an important effect on the binding affinity of the complexes with DNA [8]. Nickel plays important roles in the biology of microorganisms and plants [9]. The NiFe-hydrogenases contain nickel in addition to iron-sulfur clusters and they oxidise H_2. A nickel tetrapyrrole coenzyme, F430, is present in the methyl coenzyme reductase which powers ~methanogenic archaea. One of the carbon monoxide dehydrogenase enzymes consists of a Fe-Ni-S cluster[10]. Other nickel-containing enzymes include glyoxalase [11]. The involvement of copper in human diseases has been described from a medicinal-chemical [12] and a biochemical view [13] focusing on the molecular physiology of Cu transport [14]. Current interest in Cu complexes is stemming from their potential use as antimicrobial, antiviral, anti-inflammatory and antitumor agents, enzyme inhibitors and chemical nucleases. The biochemical action of Cu complexes with Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) was also studied[15].

Histidine (His) is involved in the coordination of copper ions in several copper proteins, including superoxide dismutase, ceruloplasmin and ascorbate oxidase. It is also involved in the transport of copper into cells from albumin. Cu^{II}-histidine complexes have been thoroughly investigated [16-18] and the structures and charge of the complexes have been defined[19-23].

Glutamic acid (Glu) can be synthesized in the body from arginine, ornithine and proline and is found in high concentration in the brain. In the body it is converted to glutamine and is the only amino acid metabolized by the brain. Glutamic acid has two carboxylate groups and it is possible to obtain structures with different kinds of carboxylate bridges[24]. L-Glutamate is a major excitatory neurotransmitter in the mammalian central nervous system that contributes not only to fast synaptic neurotransmission, but also to complex physiological processes like memory, learning, plasticity, and neuronal cell death[25-26].

Dioxane (DOX) is a polar aprotic solvent capable of acting as hydrogen bond acceptor with random structure [27]. The DOX-water mixtures are the combination of aprotic and protic solvents with wide range of dielectric constants and with good solubility for polar as well as non-polar solutes. The co-solvent-induced increased basicity of DOX-water mixtures increases the stabilization of protons. The present study is in continuation of our previous work which describes the protonation constants [28] and binary complexes[29-30].

1　Experimental

Aqueous solutions (0.1 mol·L^{-1}) of Co(II), Ni(II) and Cu(II) chlorides GR Grade (E-Merck, Germany) and 0.05 mol·L^{-1} aqueous solutions of L-histidine and L-glutamic acid (Merck, Germany) were prepared by dissolving them in triple distilled water. To increase the solubility of the ligands and metal salts, 0.05 mol·L^{-1} hydrochloric acid (Qualigens, India) was maintained in the solutions. Dioxane (Qualigens, India) was used as received. The strength of alkali was determined using the Gran plot method[31-32]. Errors in the concentrations of ligand, metal ions and alkali were subjected to analysis of variance of one way classification[33]. The titrations were carried out in the medium containing varying concentrations of the DOX maintaining an ionic strength of 0.16 mol·L^{-1} with sodium chloride (Merck, India) at 303.0 K. The measurements were made with an ELICO (Model LI-120) pH meter of 0.01 readability in conjunction with a glass and calomel electrode. The pH meter 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 DOX-water mixtures containing inert electrolyte. 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 are accounted for in the form of correction factor (lg$F$) which was computed from the experimental and simulated acid-base titration data calculated by

SCPHD program [34]. A correction was applied to the pH meter dial readings to account for the solvent effect on pH value. Titration of strong acid with alkali was carried out at regular intervals to check whether complete equilibrium was achieved. The calomel electrode was refilled with DOX-water mixtures of equivalent composition as that of the titrand. In each of the titrations, the titrand consisted of 1 mmol of hydrochloric acid (Qualigens, India) in a total volume of 50 cm³. Titrations were carried out in the presence of different relative concentrations of the metal (M) to His (L) and to Glu (X) (c_M:c_L:c_X=1:2.5:2.5, 1:2.5:5.0, 1:5.0 :2.5) with 0.4 mol·L^{-1} NaOH. The analytical concentrations of the ingredients are given in Table 1. The details of experimental procedure and titration assembly are given elsewhere[35]. The best-fit chemical model for each system investigated was arrived at using a non-linear least squares analysis program

MINIQUAD75[36].

2　Results and discussion

2.1　Modeling of chemical speciation

A preliminary investigation of alkalimetric titrations of mixtures containing different molar ratios of His and Glu in the presence of hydrochloric acid and inert electrolyte inferred that no condensed species were formed. The protonation[28] constants and the stability constants of the binary[29-30] metal complexes of these ligands were fixed in refining ternary complexes and in testing various chemical models using MINIQUAD75. The ternary complex species detected are MLXH, MLX$_2$H^{2-} and MLX$_2^{3-}$ for Ni(Ⅱ), Cu(Ⅱ) and for Co(Ⅱ) is MLX$_2$H^{2-} and MLX$_2^{3-}$ (Table 2).

A very low standard deviation (SD) in overall stability constants (lg β) indicates the precision of these parameters. The small values of U_{corr} (sum of

Table 1　Total initial concentrations of ingredients (in mmol) for mixed-ligand titrations in Dioxane water mixtures. c_{NaOH}=0.4 mol·L^{-1}; V_0=50.0 cm³; temperature: 303 K; ionic strength:0.16 mol·L^{-1}; hydrochloric acid: 1 mmol

Dioxane/% (V/V)	TM0			TL0		c_M:c_L:c_X
	Co^{Ⅱ}	Ni^{Ⅱ}	Cu^{Ⅱ}	His	Glu	
0.0	0.093 5	0.097 8	0.099	0.049 9	0.050 2	1:2.5:2.5
				0.049 9	0.050 2	1:2.5:5.0
				0.049 9	0.050 2	1:5.0:2.5
10.0	0.093 5	0.097 8	0.099	0.050 0	0.049 9	1:2.5:2.5
				0.050 0	0.049 9	1:2.5:5.0
				0.050 0	0.049 9	1:5.0:2.5
20.0	0.093 5	0.0978	0.099	0.050 0	0.050 0	1:2.5:2.5
				0.050 0	0.050 0	1:2.5:5.0
				0.050 0	0.050 0	1:5.0:2.5
30.0	0.093 5	0.097 8	0.099	0.050 0	0.050 0	1:2.5:2.5
				0.050 0	0.050 0	1:2.5:5.0
				0.050 0	0.050 0	1:5.0:2.5
40.0	0.0935	0.0978	0.099	0.050 0	0.050 0	1:2.5:2.5
				0.050 0	0.050 0	1:2.5:5.0
				0.050 0	0.050 0	1:5.0:2.5
50.0	0.093 5	0.097 8	0.099	0.049 9	0.049 9	1:2.5:2.5
				0.049 9	0.049 9	1:2.5:5.0
				0.049 9	0.049 9	1:5.0:2.5
60.0	0.093 5	0.097 8	0.099	0.049 9	0.050 1	1:2.5:2.5
				0.049 9	0.050 1	1:2.5:5.0
				0.049 9	0.050 1	1:5.0:2.5

Table 2 Best fit chemical models of His and Glu ternary complexes with Co^{II}, Ni^{II} and Cu^{II} in Dioxane-water mixtures

Dioxan /	lg β_{mlxh} (SD)			NP	$U_{corr} \times 10^8$	Skewness	Kurtosis	χ^2	R-factor	pH range
% (V/V)	MLXH	MLX$_2$	MLX$_2$H							
					Co^{II}					
00.0	—	15.60(12)	25.43(11)	94	2.55	0.86	8.26	19.11	0.009 7	4.0–10.5
10.0	—	15.48(11)	24.70(23)	92	1.24	1.18	10.41	79.09	0.006 7	4.0–10.5
20.0	—	16.57(9)	25.90(11)	76	2.55	0.08	6.22	36.42	0.008 3	4.0–10.5
30.0	—	17.28(12)	27.13(12)	99	4.32	−1.24	3.68	52.48	0.011 9	4.0–10.5
40.0	—	18.63(12)	28.46(9)	95	4.67	−1.24	4.30	36.67	0.012 0	4.0–10.5
50.0	—	18.81(10)	28.89(9)	93	2.16	−0.98	2.90	24.17	0.008 0	4.0–10.5
60.0	—	25.04(23)	35.32(16)	63	9.56	0.55	5.39	65.02	0.017 3	4.0–10.5
					Ni^{II}					
00.0	20.39(29)	17.48(22)	27.19(27)	53	1.45	0.79	3.44	10.64	0.007 1	4.4–10.0
10.0	20.55(8)	17.48(10)	26.71(25)	54	0.34	0.87	4.96	12.81	0.003 4	4.4–10.0
20.0	21.90(22)	18.67(24)	28.18(59)	30	0.90	−0.46	3.15	7.73	0.007 1	5.0–10
30.0	22.47(47)	19.73(40)	30.25(50)	37	1.54	−0.02	2.91	5.19	0.008 8	5.0–10.2
40.0	22.33(8)	19.49(14)	29.59(16)	79	0.79	−0.82	4.94	76.10	0.004 8	4.0–10.0
50.0	22.96(43)	20.77(20)	31.26(22)	51	1.70	−2.57	22.99	23.06	0.008 7	4.4–11.0
60.0	23.81(9)	26.06(11)	31.20(42)	66	0.89	−1.01	5.43	12.73	0.005 7	4.0–11.0
					Cu^{II}					
00.0	22.66(5)	21.47(17)	29.51(10)	73	0.18	0.12	3.56	14.85	0.002 1	3.0–8.5
10.0	23.11(5)	20.45(7)	30.10(9)	104	0.43	1.37	15.58	43.19	0.003 5	3.0–10.5
20.0	23.41(6)	20.34(9)	29.83(18)	108	0.42	2.71	25.83	99.11	0.003 4	3.0–10.5
30.0	24.23(8)	20.66(17)	30.26(5)	100	0.82	−0.90	4.58	38.12	0.004 5	3.0–10.0
40.0	24.86(9)	21.55(12)	31.93(14)	103	0.98	−0.28	5.00	12.23	0.005 0	3.0–10.5
50.0	25.43(9)	22.01(11)	32.11(16)	106	0.97	0.13	5.01	13.55	0.004 9	3.0–10.5
60.0	27.67(9)	31.49(19)	36.75(21)	110	5.20	0.44	3.53	16.98	0.011 0	3.0–10.0

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

squares of deviations in the concentrations of metal, ligands and hydrogen ion at all experimental points corrected for degrees of freedom) indicate that the models represent the experimental data. 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. The kurtosis values in the present study indicate that most of the residuals are greater than three and their distribution shall have sharp peak (leptokurtic) pattern in majority of the systems. The values of skewness are between −2.57 and 2.71. These data evince that the residuals form a part of normal distribution. Hence, least-squares method can be applied to the present data.

The sufficiency of the model is further evident from the low crystallographic R-values recorded.

2.2 Effect of dielectric constant on stability of ternary complexes

DOX is an aprotic coordinating solvent. It is a structure former and it enhances the water structure in DOX-water mixtures; hence, it removes water from the coordination sphere of metal ions, making them more reactive towards the ligands. As a result, the stability of the complexes is expected to increase. At the same time, it is a coordinating solvent and it competes with the ligands for coordinating the metals. This decreases the stability of the complexes. Hence, the stability of the complexes is expected to either increase or decrease linearly. The variation of lgβs with co-solvent content depends upon electrostatic and non-

electrostatic factors. Borns classical treatment[37] 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 $\lg\beta$ values should vary linearly as a function of reciprocal of the dielectric constant ($1/D$) of the medium. The non-linear variation observed in the present study (Fig.1) indicates that non-electrostatic forces are dominating the equilibrium process under the present experimental conditions.
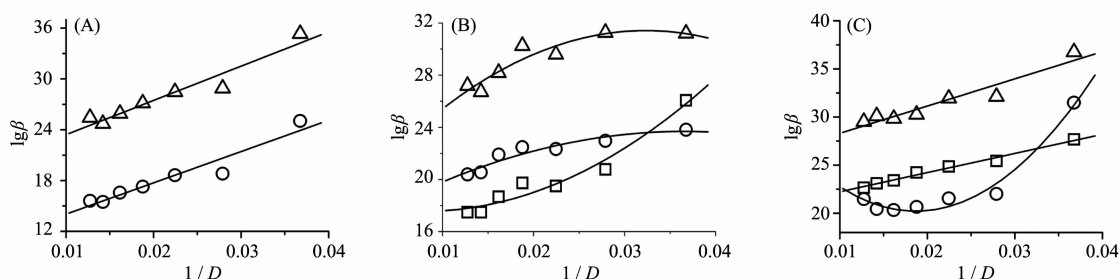

Fig.1　Variation of stability constant values of mixed-ligand ternary complexes with reciprocal of dielectric constant ($1/D$) of Dioxane-water mixtures (A) Co^{II}, (B) Ni^{II} and (C) Cu^{II} (□) $\lg\beta_{MLXH}$, (○) $\lg\beta_{MLX_2}$ and (△) $\lg\beta_{MLX_2H}$

2.3　Quantification of change in stability of species

The change in the stability of the ternary complexes as compared to their binary analogues is quantified based on the disproportionation constant[38-39] ($\lg X$) given by Equation **1**,

$$\lg X = 2\lg K_{MLX}^M - \lg K_{ML}^M - \lg K_{MX}^M \tag{1}$$

which corresponds to the equilibrium

$$ML_2 + MX_2 \rightarrow 2MLX$$
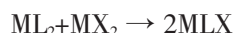
Under the equilibrium conditions one can expect the formation of 50% ternary complexes and 25% each of the binary complexes statistically and the value of $\lg X$ shall be 0.6. A value greater than this, accounts for the extra stability of MLX. Another approach to quantify the stability of ternary complexes is based on the difference in stability ($\Delta\lg K$)[40-43] for the reactions ML with X and M (aq) with L and X, where L is the primary ligand (His) and X is the secondary ligand (Glu). It is compared with that calculated purely on statistical grounds as given in Equ.**2**.

$$\Delta\lg X = \lg K_{MLX}^M - \lg K_{ML}^M - \lg K_{MX}^M \tag{2}$$

The electrostatic theory of binary complex formation and statistical arguments suggest the availability of additional coordination positions of the hydrated metal ion for the first ligand than for the second. Hence, the usual order of stability $K_{ML}^M > K_{ML_2}^{ML}$ applies. This suggests that $\Delta\lg K$ should be negative, although several exceptions have been found[44]. The statistical values of $\Delta\lg K$ for tridentate L and X are 3.54~11.53 for Co(II) and −0.90~3.56 for Cu(II) in DOX. Negative values of $\Delta\lg K$ can be understood as the secondary ligand forms a more stable complex with hydrated metal ion than with ML. Whenever the experimental values of $\Delta\lg K$ exceed the statistical values, it can be inferred that the ternary complex is formed as a result of interaction of ML with X or MX with L. The $\lg X$ and $\Delta\lg K$ values calculated from binary and ternary complexes are included in Table 3. The equations for the calculation of $\Delta\lg K$ and $\lg X$ are given in Chart 1. These values could not be calculated for some systems due to the absence of relevant binary species. In the present study, the $\lg X$ values range from 1.58 to 6.60 and some values are found to be higher than those expected on statistical bases (0.6). These higher values account for the extra stability of the ternary complexes. $\Delta\lg K$ values indicate that the ternary complexes formed by Co(II) and Cu(II) are more stable the corresponding binary complexes. The reason for the extra stability of these complexes may be due to interactions outside the coordination sphere such as the formation of hydrogen bonds between the coordinated ligands, charge neutralization, chelate effect and stacking interactions[45-46]. The extra stability of ternary complexes makes them more amenable for metal transport. The less stable binary complexes make the metals bioavailable.

Table 3 **$\Delta \lg K$ and $\lg X$ values of mixed ligand complexes of Co^{II}, Ni^{II} and Cu^{II}-His and Glu in DOX-water mixtures, calculated using the equations given below**

Dioxane / % (V/V)	$\Delta \lg K_{mlxh}$		$\lg X_{mlxh}$	
	Co^{II}	Cu^{II}	Ni^{II}	Cu^{II}
	1120	1121	1111	1111
00.0	4.50	−0.03	−0.03	1.59
10.0	3.54	0.81	0.81	2.53
20.0	4.47	−0.49	−0.49	2.07
30.0	6.00	−0.90	−0.90	2.51
40.0	6.30	0.22	0.22	2.97
50.0	4.89	−0.62	−0.62	3.04
60.0	11.53	3.56	3.56	6.59

Note: Number m, l, x, h reprensents the number of M, L, X, H, respectively

Note: $\Delta \lg K_{1111} = \lg \beta_{1111} - \lg \beta_{1101} - \lg \beta_{1010}$
$\qquad = \lg \beta_{1111} - \lg \beta_{1100} - \lg \beta_{1011}$
$\Delta \lg K_{1120} = \lg \beta_{1120} - \lg \beta_{1100} - \lg \beta_{1020}$
$\Delta \lg K_{1121} = \lg \beta_{1121} - \lg \beta_{1101} - \lg \beta_{1020}$
$\qquad = \lg \beta_{1121} - \lg \beta_{1100} - \lg \beta_{1021}$
$\lg X_{1111} = 2 \lg \beta_{1111} - \lg \beta_{1201} - \lg \beta_{1021}$
$\qquad = 2 \lg \beta_{1111} - \lg \beta_{1200} - \lg \beta_{1022}$
$\qquad = 2 \lg \beta_{1111} - \lg \beta_{1202} - \lg \beta_{1020}$
$\lg X_{1120} = 2 \lg \beta_{1120} - \lg \beta_{1200} - \lg \beta_{1040}$
$\lg X_{1121} = 2 \lg \beta_{1121} - \lg \beta_{1201} - \lg \beta_{1041}$
$\qquad = 2 \lg \beta_{1121} - \lg \beta_{1200} - \lg \beta_{1042}$
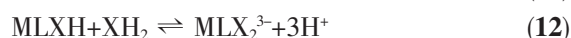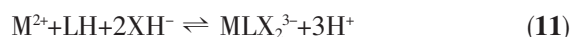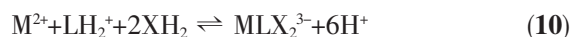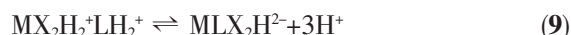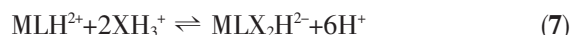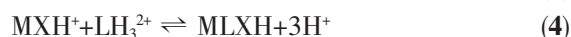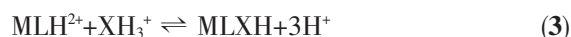$\qquad = 2 \lg \beta_{1121} - \lg \beta_{1202} - \lg \beta_{1040}$

2.4　Effect of influential parameters on stability constants

Any variation in the parameters like concentrations of ingredients affects the magnitudes of equilibrium constants. Such parameters are called influential parameters. 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 concentrations of alkali, acid, metal and ligand. The results of typical samples given in Table 4 emphasize that the errors in the concentrations of alkali and acid affect stability constant more than those of the ligands and metal.

2.5　Distribution diagrams

The mixed-ligand complex species of His (L) and Glu (X) in DOX-water mixtures are MLXH, MLX$_2$H$^-$ and MLX$_2^{2-}$. The active forms of these ligands [28] are LH$_3^{2+}$, LH$_2^+$, LH and L$^-$ and XH$_3^+$, XH$_2$, XH$^-$ and X^{2-}. The binary complex species [29-30] of His are ML$_2$H$_4^{4+}$, ML$_2$H$_2^{2+}$, ML$_2$H$^+$, MLH^{2+} and ML$_2$ for Co(II), ML$_2$H$_4^{4+}$, ML$_2$H$_2^{2+}$, ML$_2$H$^+$ and ML$_2$ for Ni(II) and ML$_2$H$_2^{2+}$, ML$_2$H$^+$, MLH^{2+} and ML$_2$ for Cu(II). In the case of Glu, the binary complexes are MX$_2$H$_2$, MX$_2$H$^-$, MX and MX$_2^{2-}$ for Co(II), MX$_2$H$_2$, MX and MX$_2^{2-}$ for Ni(II) and MX$_2$H$_2$, MX$_2$H$^-$, MXH$^+$ and MX$_2^{2-}$ for Cu(II). The formations of the ternary species are shown by the following equilibria.

$$M^{2+} + LH_3^{2+} + XH_3^+ \rightleftharpoons MLXH + 5H^+ \qquad (1)$$
$$M^{2+} + LH_2^+ + XH_2 \rightleftharpoons MLXH + 3H^+ \qquad (2)$$
$$MLH^{2+} + XH_3^+ \rightleftharpoons MLXH + 3H^+ \qquad (3)$$
$$MXH^+ + LH_3^{2+} \rightleftharpoons MLXH + 3H^+ \qquad (4)$$
$$M^{2+} + LH_3^{2+} + 2XH_3^+ \rightleftharpoons MLX_2H^{2-} + 8H^+ \qquad (5)$$
$$M^{2+} + LH_2^+ + 2XH_2 \rightleftharpoons MLX_2H^{2-} + 5H^+ \qquad (6)$$
$$MLH^{2+} + 2XH_3^+ \rightleftharpoons MLX_2H^{2-} + 6H^+ \qquad (7)$$
$$MXH^+ + LH_3^{2+} + XH_3^+ \rightleftharpoons MLX_2H^{2-} + 6H^+ \qquad (8)$$
$$MX_2H_2^+ LH_2^+ \rightleftharpoons MLX_2H^{2-} + 3H^+ \qquad (9)$$
$$M^{2+} + LH_2^+ + 2XH_2 \rightleftharpoons MLX_2^{3-} + 6H^+ \qquad (10)$$
$$M^{2+} + LH + 2XH^- \rightleftharpoons MLX_2^{3-} + 3H^+ \qquad (11)$$
$$MLXH + XH_2 \rightleftharpoons MLX_2^{3-} + 3H^+ \qquad (12)$$
$$MLX_2H_2^- \rightleftharpoons MLX_2^{3-} + H^+ \qquad (13)$$

Distribution diagrams drawn using the formation constants of the best fit model are shown in Fig.2 which contains protonated and unprotonated species. MLXH is formed by the reaction of free metal ion (FM) with protonated ligands (Equilibria **1** and **2**). MLX$_2$H^{2-} is formed by the interaction of FM with one LH$_3^{2+}$ species and two XH$_3^+$ species or LH$_2^+$ and XH$_2$ (Equilibria **5** and **6**). MLX$_2^{3-}$ is formed by the reaction

Table 4　Effect of errors in influential parameters on His-Cu^{II}-Glu complex in 20% (*V/V*) Dioxan-water mixture

Ingredient	Error / %	lgβ_{mlxh} (SD)		
		1111	1120	1121
	0	23.41(6)	20.34(9)	29.83(18)
	−5	25.17(12)	27.83(22)	33.34(22)
Acid	−2	24.14(8)	22.54(11)	31.78(12)
	+2	Rejected	Rejected	29.21(44)
	+5	Rejected	Rejected	30.54(41)
	−5	Rejected	Rejected	31.15(51)
Alkali	−2	Rejected	Rejected	30.09(30)
	+2	24.05(11)	22.98(15)	31.79(16)
	+5	24.93(22)	27.91(39)	33.01(46)
	−5	23.20(17)	20.81(12)	29.99(26)
His (L)	−2	23.33(9)	20.55(10)	29.93(21)
	+2	23.47(5)	20.10(10)	29.67(21)
	+5	23.58(7)	19.68(21)	19.67(21)
	−5	23.39(13)	21.49(9)	30.54(17)
Glu (X)	−2	23.40(8)	20.89(7)	30.24(14)
	+2	23.41(6)	19.23(10)	Rejected
	+5	23.50(9)	Rejected	29.54(13)
	−5	23.34(7)	20.31(9)	29.77(19)
Volume	−2	23.39(7)	20.33(9)	29.80(19)
	+2	23.43(6)	20.35(8)	29.86(18)
	+5	23.47(6)	20.37(9)	29.89(17)
	−5	23.44(7)	20.42(9)	29.94(16)
lgF	−2	23.42(7)	20.37(9)	29.87(18)
	+2	23.40(6)	20.31(9)	29.79(19)
	+5	23.37(6)	20.25(9)	29.72(20)
	−2	23.46(6)	20.58(7)	30.19(12)
Metal	−5	23.56(6)	20.91(7)	30.63(9)
	+5	23.28(9)	19.81(7)	Rejected
	+2	23.35(8)	20.10(11)	29.35(37)

Note: Number *m*, *l*, *x*, *h* reprensent the number of M, L, X, H, respectively

(A) Co^{II}, (B) Ni^{II} and (C) Cu^{II}
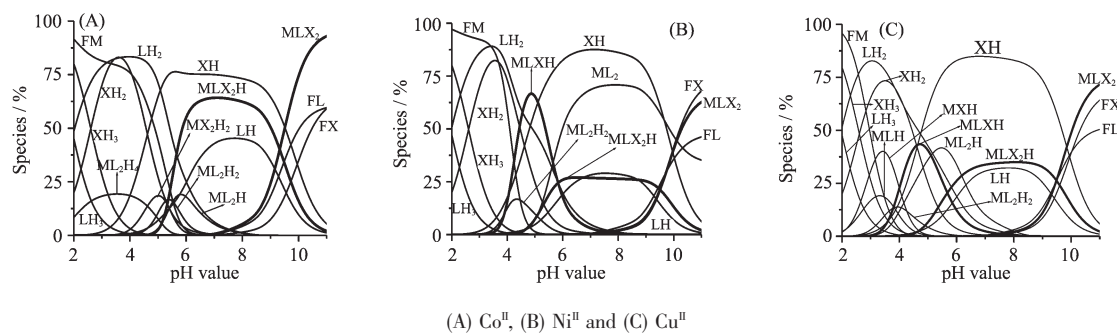
Fig.2　Distribution diagrams of mixed-ligand complexes of His and Glu in 20% (*V/V*) Dioxan-water mixture

of FM with LH and XH$^-$ or by the deprotonation of MLX$_2$H^{2-} species (Equilibria **11** and **13**). In the case

of Cu(Ⅱ) system MLXH species is formed by the interaction of FM with LH$_3^{2+}$ and XH$_3^+$ or LH$_2^+$ and

XH$_2$ or reaction of MLH^{2+} with XH$_3^+$ or MXH$^+$ with LH$_3^{2+}$ (Equilibria **1~4**). MLX$_2$H^{2-} species is formed by the interaction of FM with LH$_3^{2+}$ and XH$_3^+$ or LH$_2^+$ and XH$_2$ or by the reaction of MLH^{2+} with XH$_3^+$ or MXH$^+$ with LH$_3^{2+}$ and XH$_3^+$ (Equilibria **5~8**). MLX$_2^{3-}$ species is formed by the deprotonation of MLX$_2$H^{2-} (Equilibrium **14**). MLX$_2^{3-}$ species is formed at higher pH value than MLXH and MLX$_2$H^{2-}. For His-Co(II)-Glu system MLX$_2$H^{2-} is formed by the interaction of FM with LH$_3^{2+}$ and XH$_3^+$/LH$_2^+$ and XH$_2$ or by the reaction of MX$_2$H$_2$ with LH$_2^+$ (Equilibria **5**, **6** and **9**).

MLX$_2^{3-}$ species is formed by the interaction of FM with LH$_2^+$ and XH$_2$/LH and XH$^-$ or by the deprotonation of MLX$_2$H^{2-} (Equilibria **10**, **11** and **13**).

2.6 Structures of complexes

Although it is not possible to elucidate or confirm the structures of complex species pH metrically, depending upon the nature of the ligands and the metal ions and based on the chemical knowledge the tentative structures of the ternary complexes are proposed as shown in Fig.3.
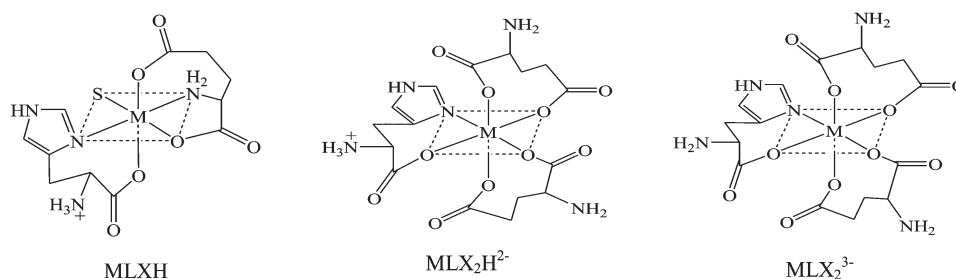

Fig.3 Structures of His-Metal-Glu complexes, where S is either solvent or water molecule

3 Conclusions

The following conclusions have been drawn from the speciation studies of mixed-ligand complexes of Co^{II}, Ni^{II} and Cu^{II} with His (L) and Glu (X) in DOX-water mixtures.

1. The mixed-ligand complex species detected are MLX$_2$H^{2-} and MLX$_2^{3-}$ for Co^{II}, MLXH, MLX$_2$H^{2-} and MLX$_2^{3-}$ for Ni^{II} and Cu^{II}.

2. The values of $\Delta \lg K$ and $\lg X$ indicate that the ternary species have extra stability compared to their binary species.

3. The non-linear increase in the stabilities of mixed-ligand complexes with decreasing dielectric constant of the medium is due to dominance of non-electrostatic forces.

4. The magnitudes of the stability constants for mixed-ligand complexes are affected by the errors in the influential parameters like the concentrations of the ingredients. The order of influence is alkali>acid> ligands>metal>ligand.

5. The value of $\Delta \lg K$ indicate that the ternary species have extra stability compared to their binary species, may be due to the interactions outside the coordination sphere, such as the formation of hydrogen bonds between the coordinated ligands, charged neutralization, chelate effect, stacking interactions and electrostatic interaction between non-coordinated charge groups of ligands.

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