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Mixed ligand complexes of essential metal ions with L-glutamine and succinic acid in sodium dodecyl sulfate–water mixtures

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Abstract: Speciation of mixed ligand complexes of Co(II), Ni(II) and Cu(II) with L-glutamine and succinic acid was studied in varying amounts (0.0–2.5 %, w/v) of sodium dodecyl sulfate (SDS) in aqueous solutions while maintaining an ionic strength of 0.16 mol L⁻¹ (NaCl) at 303.0 K. Titrations were performed in the presence of different relative concentrations (M:L:X = 1:2:2, 1:4:2 and 1:2:4) of metal (M) to L-glutamine (L) to succinic acid (X) using sodium hydroxide. The stability constants of the ternary complexes were refined with MINIQUAD75. The best-fit chemical models were selected based on statistical parameters and residual analysis. The species detected were ML₂X, MLX, MLXH and MLXH₂ for Co(II), Ni(II) and Cu(II). The increased stability of the ternary complexes compared to their binary complexes was believed to be due to electrostatic interactions of the side chains of the ligands, charge neutralization, chelate effect, stacking interactions and hydrogen bonding. The species distribution with pH at different compositions of SDS and plausible equilibria for the formation of the species are also presented.

Keywords: speciation; mixed ligand complexes; L-glutamine; succinic acid; SDS.

INTRODUCTION

The specificity and selectivity of enzyme–substrate reactions are achieved by manipulating the equivalent solution dielectric constants at the active site. Knowledge of the equivalent solution dielectric constant can throw light on the mechanism of the reaction. Furthermore, intramolecular and ligand–ligand stacking interactions in mixed ligand complexes are favored in water–organic media, which reduce the dielectric constant of the solution. Hence, modeling studies involving ternary complexes have gained popularity in different aqua–organic mixtures with varying dielectric constants.^{1–4} L-Glutamine (Gln) serves as a vehicle for transporting ammonia in a nontoxic form from peripheral tissues to visceral or-

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gans where the ammonia can be excreted as ammonium ions (kidneys) or converted to urea (liver).⁵ Gln is utilized in the brain for respiration and biosynthesis of substances related to neuronal functions, such as γ -aminobutyric acid, GABA. It is also utilized for the growth and differentiation of the neural cells.⁶ It is often depleted in stress states, such as malignancy.⁷ Gln has a possible therapeutic role in the prevention of damage to normal tissues, including peripheral nerves, during chemotherapy.⁸

Succinic acid (Suc) is involved in the citric acid or tricarboxylic acid (TCA) cycle and the glyoxalate cycle. It is synthesized in almost all microbial, plant, and animal cells.⁹ The concentration of Suc in human blood plasma is 1.0–6.0 mg L⁻¹. Suc can be used for the manufacture of medicaments or nutritional supplements effective for the treatment of insulin resistance in mammals, preferably in non-insulin dependent diabetic humans.¹⁰ On the other hand, recent analysis showed that the fermentative production of Suc from renewable resources could be more cost-effective than the petroleum-based processes.¹¹ Suc is a bidentate ligand. In coordination chemistry, it forms strong complexes with many metal ions. The mixed ligand complexes of Gln and Suc with calcium and magnesium in acetonitrile–water and ethylene glycol–water and of cobalt and nickel in urea–water and DMF–water media were reported earlier.^{1,12–14} In this study, speciation of ternary complexes of Co(II), Ni(II) and Cu(II) with Gln and Suc in SDS–water mixtures was investigated.

EXPERIMENTAL

Solutions of Co(II), Ni(II) and Cu(II) chlorides (0.1 mol L⁻¹ were prepared by dissolving GR Grade (Merck, India) salts in triple distilled water. Aqueous solutions of Gln and Suc (Merck, India) were also prepared. To increase the solubility of Gln and Suc and to suppress hydrolysis of the metal salts, the mineral acid concentration in the above solutions was maintained at 0.05 mol L⁻¹. SDS (Merck, India) was used as received. A 0.2 mol L⁻¹ hydrochloric acid (Qualigens, India) solution and a 0.4 mol L⁻¹ sodium hydroxide (Qualigens, India) were prepared. A 2 mol L⁻¹ sodium chloride (Qualigens, India) solution was prepared to maintain the ionic strength in the titrand. 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 of the alkali was determined using the Gran plot method.¹⁵

Apparatus

The titrations were performed in media containing varying concentrations of SDS maintaining an ionic strength of 0.16 mol L⁻¹ with sodium chloride at 303.0±0.1 K. A systronics μ pH system (model 362) (readability 0.001) was used. The glass electrode was equilibrated in a well-stirred SDS–water mixture containing inert electrolyte. The effect of variations in asymmetry, 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 a correction factor, which was computed from simulated acid–base titration data calculated by the SCPHD program.^{16,17} A correction was applied to the pH meter dial reading by using a correction factor. Titrations of a strong acid with alkali were performed at regular intervals to check the complete equilibration of the electrode. The calomel electrode was refilled with an SDS–water

mixture of equivalent composition to that of the titrand. In each of the titrations, the titrand consisted of 1–3 mmol of mineral acid in a total volume of 50 cm³. The titrations were performed in the presence of different relative concentrations (1:2:2, 1:4:2 and 1:2:4) of metal (M) to Gln (L) and Suc (X) with 0.4 mol L⁻¹ NaOH.

Modeling strategy

The best-fit chemical models consisting of stoichiometric coefficients and logarithm of stability constants ($\log \beta$) were arrived at using the computer program Miniquad 75.¹⁸ Some heuristics were followed in refining stability constants and validating models.^{19,20} The formation constants for acid–base equilibria and those for binary metal complexes of Gln and Suc were fixed in the refinement of the mixed ligand stability constants using MINIUAD75.

RESULTS AND DISCUSSION

Complex equilibria

A preliminary investigation of alkalimetric titrations of mixtures containing different mole ratios of Gln and Suc in the presence of a mineral acid and an inert electrolyte inferred that MLXH, MLX, ML₂X and MLXH₂ species are formed for Co(II), Ni(II) and Cu(II). The parameters of the best-fit models and statistical parameters are given in Table I.²¹ The very low standard deviation in the $\log \beta$ values indicates the precision of these parameters. The small values of U_{corr} indi-

TABLE I. Best fit chemical models of Co(II), Ni(II) and Cu(II)–L-glutamine–succinic acid complexes in SDS–water mixtures. Temperature: 303 K; ionic strength: 0.16 mol L⁻¹

SDS % w/v	$\log \beta_{\text{mlxh}} (SD)$				NP	$U_{\text{corr}} \times 10^8$	Skew- ness	Kurto- sis	χ^2	R Factor
	1110	1111	1112	1210						
Co(II) (pH range 2.0–9.0)										
0.0	8.12(13)	14.13(30)	18.99(21)	11.88(10)	152	14.27	1.40	15.43	169.24	0.013
0.5	7.94(23)	14.85(15)	18.85(39)	11.90(13)	177	25.05	4.50	43.64	416.27	0.016
1.0	7.69(25)	14.23(30)	18.54(47)	11.65(13)	177	28.64	3.62	35.50	333.20	0.018
1.5	7.78(29)	14.47(29)	19.17(21)	11.85(11)	182	18.93	7.15	76.51	744.57	0.014
2.0	7.78(26)	14.05(52)	19.26(17)	11.81(11)	177	28.14	3.82	37.05	236.59	0.017
2.5	7.15(25)	14.15(14)	18.82(12)	11.06(12)	182	14.13	6.28	64.73	593.91	0.012
Ni(II) (pH range 2.0–9.0)										
0.0	9.09(19)	14.71(20)	19.79(10)	13.40(13)	168	18.03	-0.71	6.06	136.79	0.014
0.5	8.80(16)	14.17(24)	18.66(20)	13.00(11)	168	11.15	-0.09	12.45	313.38	0.011
1.0	8.79(13)	13.99(34)	18.68(19)	13.02(12)	167	16.09	-0.81	10.55	323.43	0.013
1.5	8.06(14)	13.83(23)	18.80(11)	11.95(20)	176	17.95	-1.01	8.50	289.27	0.013
2.0	8.14(15)	13.73(26)	18.69(12)	12.27(16)	173	20.39	-0.37	8.17	173.85	0.015
2.5	7.99(13)	13.51(24)	18.46(9)	11.66(44)	181	10.92	0.38	8.30	40.02	0.010
Cu(II) (pH range 2.0–6.0)										
0.0	11.73(7)	16.33(8)	20.13(10)	17.79(7)	153	2.73	-0.60	3.80	23.08	0.005
0.5	11.69(6)	15.99(6)	19.67(8)	17.58(6)	149	4.68	-1.69	9.91	138.36	0.007
1.0	11.18(8)	15.78(4)	19.23(7)	17.97(4)	161	4.21	-0.55	7.71	74.41	0.006
1.5	12.40(9)	16.39(14)	20.59(7)	18.07(13)	150	15.08	0.03	4.75	53.41	0.012
2.0	11.03(16)	15.29(19)	19.52(7)	17.31(7)	157	6.38	-0.91	5.67	29.30	0.008
2.5	12.30(8)	15.75(28)	19.98(8)	18.31(11)	156	11.48	-0.24	3.20	1.69	0.010

cate that the model is consistent with the experimental data.²² For most of the systems, the kurtosis values were around 3 and, hence, the residuals form a mesokurtic pattern. For very few systems are the kurtosis values more than 3 (leptokurtic pattern). The values of skewness between -1.69 and 7.15 show that the residuals form a part of a normal distribution and 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 -factor values, which indicate the need for inclusion of additional species in the model. χ^2 is a special case of a γ -distribution that measures the probability of residuals forming a part of standard normal distribution.

The linear variation (Fig. 1) of the stability constants of the Gln and Suc complexes of Co(II), Ni(II) and Cu(II) in SDS–water mixtures with mole fraction indicates that electrostatic forces dominate the equilibrium process under the employed experimental conditions. The stability of a complex depends on the charge of the Stern layer,²³ polarity of the medium and the electrostatic attraction or

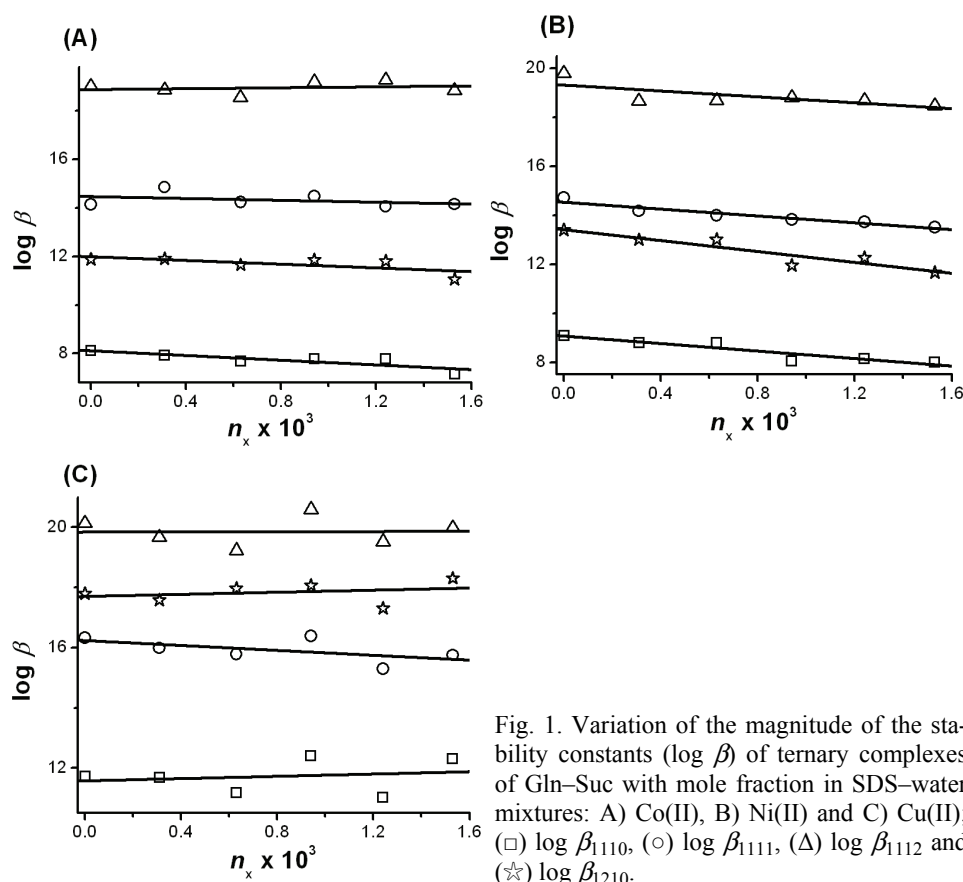


Fig. 1. Variation of the magnitude of the stability constants ($\log \beta$) of ternary complexes of Gln–Suc with mole fraction in SDS–water mixtures: A) Co(II), B) Ni(II) and C) Cu(II); (\square) $\log \beta_{1110}$, (\circ) $\log \beta_{1111}$, (Δ) $\log \beta_{1112}$ and (\star) $\log \beta_{1210}$.

repulsive forces operating between the complex species and the micellar surface. This linear decrease²⁴ is due to the decreasing dielectric constant of the medium with increasing surfactant content.²⁵ The charged species will be destabilized due to the decreased dielectric constant of the medium with increasing surfactant concentration. The stability of the neutral mixed complexes (Fig. 1) is not influenced by SDS. SDS acts as a denaturant of macromolecules by interacting with the peptide groups. Hence, the stability of the species decreases with increasing SDS content. Another reason for the decreased stability of the ternary complexes is the ligation power of SDS.

Stability of the ternary complexes

The change in the stability of the ternary complexes as compared to their binary analogues was quantified.^{26,27} In one of the approaches, the difference in stability ($\Delta \log K$) for the two reactions ML with X and M(aq) with X is compared with that calculated purely on statistical grounds. Eq. (1) can be formulated based on the properties of the cyclic systems reported earlier, from which it is clear that both the ligands in the ternary complex mutually influence to the same extent.^{28,29}

$$\begin{aligned} \Delta \log K &= \log K_{MLX}^{ML} - \log K_{MX}^M = \log K_{MLX}^{MX} - \log K_{ML}^M = \\ &= \log K_{MLX}^M - \log K_{ML}^M - \log K_{MX}^M \end{aligned} \quad (1)$$

Both the electrostatic theory of binary complex formation and statistical arguments clearly indicate that, in the case of a given multivalent hydrated metal ion, more coordination positions will be available for the first ligand than for the second one. Hence, the order of stability $K_{ML}^M > K_{ML_2}^{ML}$ holds well. This leads to the natural expectation for $\Delta \log K$ to be negative, although several exceptions have been found.^{26,30} The statistical values of $\Delta \log K$ for bidentate L and X are -0.4 and -0.6 for octahedral and square planar complexes, respectively, whereas for distorted octahedral complexes, the values vary between -0.9 and -0.3 . Negative values of $\Delta \log K$ can be understood as the secondary ligand forms a more stable complex with the hydrated metal ion than with ML, which does not mean that the ternary complex is absent.

Whenever the experimental value of $\Delta \log K$ exceeds the statistical value, it can be inferred that the ternary complex is formed as a result of interaction of ML with X or MX with L. Sigel postulated that $\Delta \log K$ values of ternary complexes containing bipyridyl as the primary ligand were positive for *O*-donors (malonic acid, pyrocatechol, etc.), negative for *N*-donors (ethylene diamine) and intermediate or many times a small negative values for amino acids with both N and O coordination sites.^{26,31,32} However, a very high negative value (-2.3) for Cu(en)(iminodiacetic acid) and a positive value (0.82) in the case of Cu(ophen)-(6,7-dihydroxynaphthalene-2-sulfonate) were also observed.²⁶

The formulae for the calculation of $\Delta \log K$ values are given in Table II. The $\Delta \log K$ values calculated for the binary and ternary complexes are incorporated in Table II. Co(II), Ni(II) and Cu(II) ions form octahedral complexes with Gln and Suc. For most of the systems given in Table II, the values of $\Delta \log K$ are found to be higher than those expected on statistical grounds (-0.4). The increased stability of the ternary complexes might be due to the interactions outside the coordination sphere, such as the formation of hydrogen bonds between the coordinated ligands. In addition, a similar stabilizing effect may likewise be exerted by electrostatic interactions between non-coordinated, charged groups of the ligands, such as the $-\text{NH}_3^+$ of Gln and the $-\text{COO}^-$ of Suc.²

TABLE II. Variation of stability of ternary complexes of Gln–Suc in SDS–water mixtures. $\Delta \log K_{1110} = \log \beta_{1110} - \log \beta_{1100} - \log \beta_{1010}$; $\Delta \log K_{1111} = \log \beta_{1111} - \log \beta_{1100} - \log \beta_{1011}$; $\Delta \log K_{1112} = \log \beta_{1112} - \log \beta_{1101} - \log \beta_{1011}$; $\Delta \log K_{1210} = \log \beta_{1210} - \log \beta_{1200} - \log \beta_{1010}$; $\log X_{1110} = 2 \log \beta_{1110} - \log \beta_{1200} - \log \beta_{1020}$

SDS, % w/v	$\Delta \log K_{1110}$	$\Delta \log K_{1111}$	$\Delta \log K_{1112}$	$\Delta \log K_{1210}$	$\log X_{1110}$
Co(II)					
0.0	1.1	1.87	0.06	1.36	2.72
0.5	0.72	2.7	-0.42	0.68	1.93
1.0	2.38	3.77	1.72	2.56	3.33
1.5	-0.54	1.78	-0.34	-0.04	1.02
2.0	1.17	3.33	1.87	1.32	2.57
2.5	0.47	3.22	1.19	0.29	0.49
Ni(II)					
0.0	0.84	1.56	1.0	0.37	2.52
0.5	1.89	1.93	0.41	1.12	2.88
1.0	0.83	1.06	0.19	0.48	1.93
1.5	0.49	1.56	1.93	-0.38	1.47
2.0	–	1.74	0.73	–	1.42
2.5	1.26	2.06	0.99	-0.43	0.82
Cu(II)					
0.0	-0.47	-0.58	-0.15	-1.16	1.85
0.5	–	0.38	0.39	–	2.97
1.0	–	1.42	1.4	–	3.5
1.5	1.78	1.44	2.07	0.78	4.48
2.0	-0.77	-0.55	0.07	-1.1	0.34
2.5	1.8	0.72	1.61	0.57	3.56

Effect of influential parameters on stability constants

Any variation in the parameters (the concentrations of the chemicals) affects the magnitudes of the equilibrium constants. Such parameters are called influential parameters. In order to rely on 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 pessi-

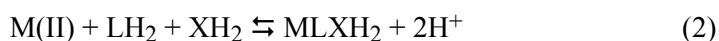
mistic errors in the concentrations of alkali, acid, ligands, metal ions and correction factor. The results in Table III emphasize that the errors in the concentrations of alkali and acid affect the stability constant more than the errors of the ligands and the metal ion.

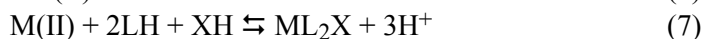
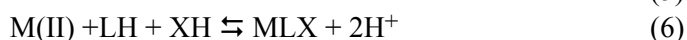
TABLE III. Effect of errors in the influential parameters on stability constants of ternary complexes of Ni(II) with Gln–Suc in a 1.5 % w/v SDS–water mixture

Component	Error, %	$\log \beta (SD)$			
		1110	1111	1112	1210
Alkali	0	8.06 (14)	13.83 (23)	18.80 (11)	11.95 (20)
	–5	Rejected	Rejected	19.55 (12)	Rejected
	–2	7.15 (41)	Rejected	18.92 (08)	Rejected
	+2	9.54 (12)	15.25 (12)	19.71 (9)	13.99 (16)
	+5	10.99 (18)	16.14 (18)	20.30 (13)	16.06 (24)
Acid	–5	10.79 (13)	16.34 (12)	20.59 (9)	15.39 (16)
	–2	9.46 (12)	15.32 (11)	19.88 (8)	13.68 (15)
	+2	7.33 (24)	Rejected	18.63 (9)	Rejected
	+5	Rejected	Rejected	18.84 (12)	Rejected
Gln	–5	7.90 (15)	13.50 (36)	18.57 (13)	11.98 (17)
	–2	7.99 (14)	13.69 (27)	18.70 (12)	11.95 (19)
	+2	8.14 (13)	13.97 (20)	18.91 (10)	11.96 (21)
	+5	8.33 (13)	14.23 (16)	19.13 (9)	12.07 (21)
Suc	–5	8.80 (13)	14.38 (15)	18.87 (14)	13.23 (16)
	–2	8.36 (13)	14.09 (17)	18.80 (12)	12.53 (16)
	+2	7.71 (17)	13.41 (45)	18.84 (10)	Rejected
	+5	7.68 (19)	13.86 (23)	19.24 (8)	Rejected
Metal	–5	8.18 (14)	14.02 (19)	18.90 (11)	12.24 (17)
	–2	8.10 (14)	13.90 (21)	18.84 (11)	12.06 (19)
	+2	8.02 (14)	13.75 (25)	18.77 (11)	11.82 (22)
	+5	7.96 (14)	13.65 (28)	18.73 (11)	11.62 (26)

Distribution diagrams

A perusal of the distribution diagrams (Fig. 2) reveals that at very low pH values, the concentrations of the mixed ligand complexes are lower than those of protonated ligands. The concentrations of the ternary species increased with increasing pH. The protonated ternary species, MLXH and MLXH₂ are distributed at lower pH values and the unprotonated ternary species, MLX and ML₂X at higher pH values. Higher concentrations of the ternary species than those of binary species indicate the higher stability of the former. The ternary species exist in the pH range 2.0–9.0 for Co(II), Ni(II) and 2.0–6.0 for Cu(II), respectively. The formation of the complex species can be represented by the following equilibria. The charges of the species are omitted for clarity.





The protonated ligands LH_2 and XH_2 interact with the metal ion to form MLXH_2 (Eq. (2)), which successively loses protons to form MLXH (Eq. (3)) and MLX (Eq. (5)). MLXH species may also be formed due to (Eq. (4)). LH and XH interact with the metal ion to form MLX (Eq. (7)). ML_2X is formed by (Eq. (6)). MLXH_2 may be formed by the interaction of MXH and LH_2 (Eq. (8)) and/or MLH and XH_2 (Eq. (9)). MXH and LH interact to form MLXH (Eq. (10)). The possible structures of these ternary complexes are proposed in Fig. 3. The exis-

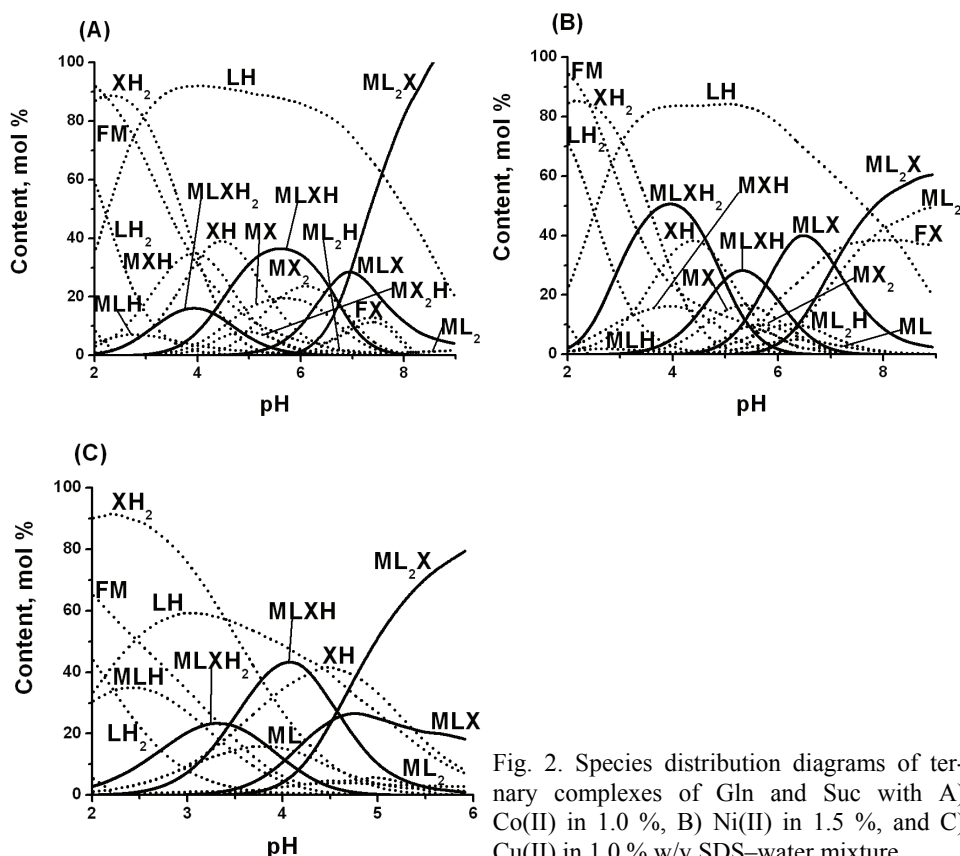


Fig. 2. Species distribution diagrams of ternary complexes of Gln and Suc with A) Co(II) in 1.0 %, B) Ni(II) in 1.5 %, and C) Cu(II) in 1.0 % w/v SDS-water mixture.

tence of ML_2X and the absence of MLX_2 are due to the higher affinity of LH than XH towards the metal ion.

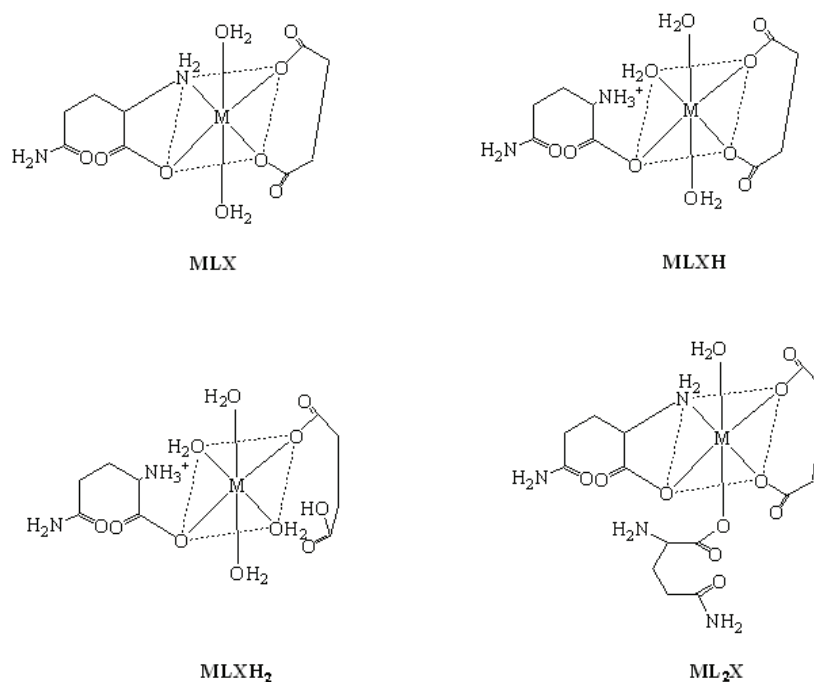


Fig. 3. Structures of ternary complexes of Co(II), Ni(II), Cu(II) with Gln and Suc.

Biological relevance of the present study

The presence of SDS considerably decreases the dielectric constant of the aqueous solution and creates compartmentalization due to the formation of micelles. Thus, the surfactant solution is expected to mimic the physiological conditions of the active site cavities with low dielectric constants and the concept of equivalent solution dielectric constant in the active site cavities is invoked. The stability constants determined and the species predicted under these mimicked conditions can be extrapolated to those expected to be present in the active site cavities of biomolecules. Such studies are useful to understand: *i*) the role played by the active site cavities in biological molecules, *ii*) the type of complex formed by the metal ion, *iii*) the bonding behavior of protein residues with a metal ion, and *iv*) the greater significance of the concentrations of complex chemical species than the total concentrations of essential metals in soils and water in predicting their bioavailability. The species refined and their relative concentrations under the present experimental conditions represent the possible forms of Co(II),

Ni(II) and Cu(II) ions in biological fluids and the speciation represents the biological activity of these metals in the presence of glutamine and succinate residues.

CONCLUSIONS

The following conclusions have been drawn from the modeling studies of the speciation of ternary complexes of Co(II), Ni(II) and Cu(II) with Gln and Suc in SDS–water media.

The species detected were MLX, MLXH, MLXH₂ and ML₂X for Co(II), Ni(II) and Cu(II).

The linear variation of the log β values with mole fraction of the medium indicates the dominance of electrostatic forces over non-electrostatic forces.

The change in the stability of the ternary complexes as compared to their binary analogues shows that the ternary complexes are more stable than the binary complexes due to the interactions outside the coordination sphere.

The existence of ML₂X and the absence of MLX₂ are due to the higher affinity of LH than XH towards the metal ion.

This study also gives an insight into the bioavailability/bioaccumulation of these metals. The ternary complexes are more amenable for metal transport because of their extra stability while the binary complexes make the metal bioavailable due to their decreased stability.

ИЗВОД

ФОРМИРАЊЕ КОМПЛЕКСА ЕСЕНЦИЈАЛНИХ ЈОНА МЕТАЛА СА МЕШОВИТИМ ЛИГАНДИМА L-ГЛУТАМИНОМ И ЋИЛИБАРНОМ КИСЕЛИНОМ У СМЕШИ НАТРИЈУМ-ДОДЕЦИЛ-СУЛФАТА (SDS) И ВОДЕ КАО РАСТВАРАЧА

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У различитим односима натријум-додецил-сулфата и воде као растварача (0,0–2,5%, m/v) и јонској јачини од 0,16 mol L⁻¹ (NaCl), на температури од 303,0 K, изучавано је формирање комплекса Co(II), Ni(II) и Cu(II) са мешовитим лигандима L-глутамином и ћилибарном киселином. Титрације су вршене раствором натријум-хидроксида при различитим односима концентрација метала (M), L-глутамин (L) и ћилибарне киселине (X) (M:L:X = 1:2:2, 1:4:2 и 1:2:4). Константе стабилности су израчунате помоћу Miniquad 75 програма, док је најбоље слагање хемијских модела добијено на основу статистичких параметара и методом резидуалних анализа. Нађено је да у раствору постоје комплекси са следећим општим формулама: ML₂X, MLX, MLXH и MLXH₂ за Co(II), Ni(II) и Cu(II). Већа стабилност тернарних у односу на одговарајуће бинарне комплексе се објашњава електростатичким интеракцијама бочних ланаца лиганата, хелатним ефектом, неутрализацијом наелектрисања, као и постојањем одређених интеракција налегања и водоничних веза.

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